SPECIAL TUBERCULOSIS NUMBER

# BULLETIN

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JUL 25 1911

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Vol. XXII.—No. 245.] BALTIMORE, JULY, 1911. [Price, 50 Cents

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## THE STEREOSCOPIC X-RAY EXAMINATION OF THE CHEST WITH ESPECIAL REFERENCE TO THE DIAGNOSIS OF PULMONARY TUBERCULOSIS.

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### PART I.

By H. Kennon Dunham, M. D.

The object of this paper is to call attention to the principles and technique of stereoscopic X-ray examinations, and to describe the alterations in the normal chest shadows, which we believe to be characteristic of pulmonary tuberculosis.

The X-ray, when properly applied, is an instrument of precision for recording differences of density. On the single negative we have of necessity various shadows superimposed. In stereo-rœntgenographs taken with mathematical precision the chest is seen in true perspective, the shadows occupying the same relative position in the image, as the structures which cast the shadows occupy in the chest. This result is obtained by applying the principles of binocular vision. As you will recall "the retinal images of external objects, particularly near objects, are different in the two eyes. Whenever what we may call a right-eyed image of an object is thrown on the right eye and simultaneously, a left-eyed image is thrown on the left eye, whether in nature or by an artifice, we at once perceive depth or solidity in the object." The technical problem then, in stereo-rœntgenography was, first to obtain two

negatives corresponding to the right-eyed and to the left-eyed image respectively, and second, to view simultaneously, by means of some suitable optical device, the right-eyed image with the right eye and the left-eyed image with the left eye.

There are at present several types of apparatus which enable us to obtain the necessary negatives. The common principles in these are, first, a movable carrier for the X-ray tube, and second, a movable carrier for the X-ray plates.

In the movement of the tube there are four details requiring consideration. First, recalling that the X-rays originate at the so-called target of the tube, this target should be moved in a plane parallel to the X-ray plate, in order that the focal distance may be equal in both exposures. Second, between the first and second exposures the target should be moved a distance slightly less than the inter-pupillary distance. Six centimeters has been found satisfactory. Thus every one who has good use of both eyes can see the stereo picture and persons with a greater pupillary distance can estimate, by means of their personal equation, the amount of fore-shortening and allow for it. Third, the target should be moved in a line parallel to rather than across the spine of the patient because this enables us to see around the ribs, thereby obtaining a less obstructed view of the lung fields. Fourth, the target should not be too near the plate. Twenty-five inches is a good working average.

As to the movement of the plates it is merely necessary that the second plate on exposure occupy the position held by the first plate during its exposure. Finally, the total time required for the two exposures, and the movement of tube and plates must not exceed a period of easily sustained inspiration, a time well under ten seconds. For observing the finished plates, the stereoscope devised by Whetstone early in the last century, is most generally used.

Turning now to a study of the shadow pictures seen in stereo-rœntgenographs of human chests, let us first consider the normal before attempting to describe the tuberculous.

On examining a stereo-rœntgenograph of a normal thorax, one sees the shadow picture of the bony framework, with its covering of soft parts, enclosing the chest cavity, except below, where it is bounded by the shadow of the diaphragm. The shadows within the chest cavity, seen as we have stated, in their normal relations, are readily divided into three main groups: first, the "heavy central shadow," extending irregularly from the upper boundary of the chest cavity to the diaphragm; second, the "hilus shadow," radiating irregularly to a distance of four or five centimeters on either side of the fifth, sixth and seventh dorsal vertebræ; and third, the "linear markings" seen in the lung fields.

The "heavy central shadow" need not here be described in detail. It is cast by the vertebræ and the mediastinal contents; the heart, aorta and other large vessels, trachea, œsophagus, lymphatics and connective tissue. The trachea can usually be distinguished as a band of less density bordered by parallel bands of great density, extending downward from the neck. Often it can be seen to divide in front of the fifth dorsal vertebra, the shadow of one bronchus going to the right, and the other to the left hilus. The right is the more often seen as the left passes under the aortic arch and is generally obscured for some distance. In good plates, these bronchial shadows may be seen to subdivide and extend into the lung fields, as a part of the heavy trunks, to be described directly.

The hilus shadow, as has been stated, is cast by the primary branches of the pulmonary vessels with their contained blood, the walls of the primary bronchi, and the lymphatic and fibrous tissue surrounding these structures. Normally, the hilus shadow is of moderate density, irregular in outline and of small extent, merging internally with the heavy central shadow. Externally the hilus has a sharp border which is made irregular by the heavy trunks emerging from it into the lung fields.

The shadows in the lung fields may be subdivided into two groups: the heavy trunks and the fine linear markings: The heavy trunks appear as definite shadows radiating from the hilus shadow toward the periphery. Three groups can usually be seen on the right side, one group running upward and outward, another outward and the third downward and outward. These groups mark what we call divisions. They correspond roughly to the lobes of the lung. On the left side only two such groups can be seen, one extending upward and outward, the other downward and outward. Distally these heavy trunks divide and subdivide producing the radiating shadows designated as the fine linear markings. Examined more carefully, these fine radiating shadows appear as fairly definite straight lines extending toward the periphery, but with our technique, they are lost on the normal plate before they reach the periphery of the lung. These shadows are the combined shadows cast by the blood vessels with their contained blood, the walls of the bronchi, reinforced by the accompanying lymphatics and fibrous tissue. The heavy trunks are the shadows of the large branches distributed to separate lobes of the lung, the linear markings are the shadows cast by the smaller branches distributed to the various lobules of the separate lobes. The linear markings in the lower left division of the lung are rarely distinct, owing to movement, during exposure, occasioned by the cardiac contractions. Therefore alterations in the character of the markings in this region cannot be accurately determined.

Bearing in mind, then, this normal shadow picture, let us consider the stereo-rœntgenographs of chests in which early but definite signs of pulmonary tuberculosis are found on physical examination. In such cases changes are noted in the hilus shadow, the heavy trunks and especially in the linear markings.

The hilus shadow shows usually an irregular increase in area and density with here and there small irregular shadows of very marked density. These bright areas of great density are due to the presence in the chest of calcified caseous or fibrous glands, while the increased area and density of the hilus shadow is due to a general increase in the fibrous and lymphatic tissue which accompanies a local or general mediastinitis. Such changes alone are of very slight diagnostic value as re-

gards the presence of active tuberculosis, since other infections may produce the mediastinal changes and the calcified glands, although probably of tuberculous origin, indicate an old process more or less healed. In fact the presence of calcified glands, the other conditions being favorable, may be taken as a good prognostic sign.

The heavy trunks extending toward the involved area usually appear broader, denser and less regular in outline than in the normal. Here again this change in the heavy trunks is not necessarily tuberculous in origin, so that too great importance must not be attached to this change alone.

Accompanying this alteration in the heavy trunks we find in the diseased area a similar change affecting the fine linear markings. They appear broader, denser and less regular in outline, frequently their course is studded, sometimes almost to obliteration of the lines. We can no longer trace them as radiating but find them broken in continuity and reaching to or near the periphery. As a result of these changes the linear markings appear to cross and interweave, producing a delicate network of varying sized mesh. In the uninvolved areas of the lung the shadow picture may be normal or there may be a slight alteration, such as thickening or increased density of the trunks and linear markings. The anatomical explanation of this altered shadow picture we are not at this time ready to discuss, but we firmly believe that the increase in the hilus shadow, the thickening of the trunks, together with the alterations in the linear markings—increase in density and breadth, studding, interweaving and extension to the periphery—constitute a shadow picture characteristic of early pulmonary tuberculosis. As the lesion progresses the alterations become more marked, the linear markings are more and more broken up and irregular, the studdings become larger and denser and the interweaving closer, until eventually the whole area appears as a more or less homogenous shadow of increased density, due to the presence of gross areas of consolidation within the lung. The more advanced change due to cavity formation we need not discuss at this time.

We have now considered the appearance of the stereo-rœntgenograph of the normal chest and of the typically early tuberculous chest. However, all cases do not fall into these two classes. In the clinical report by Dr. Wolman the cases were divided into five groups: (1) Normal; (2) Diseased but not Tuberculosis; (3) Probably Tuberculosis; (4) Definitely Tuberculosis; (5) Healed Tuberculosis. It must be clearly understood that the stereo-rœntgenographic examination does not draw a sharp line of differentiation between these various groups any more than does the clinical examination. In deciding the border line cases the personal attitude and experience of the examiner is of great importance. We cannot, therefore, set forth any absolute rules to be applied in the classification of every case, but a brief description of the shadow pictures which we consider typical of the various groups may be of some assistance.

The normal picture has already been discussed. It might be mentioned that with advancing age there is an increase in the density of all the shadows, hilus, heavy trunks, and linear markings, for which due allowance must be made.

There are many non-tuberculous pulmonary lesions, but we will not at this time discuss the stereo-rœntgenographic appearance of acute pneumonia, abscess, gangrene, cyst, etc., except to say that they should cause no diagnostic difficulty. There is, however, a large class of cases presenting indefinite clinical signs suggesting pulmonary tuberculosis, which upon examination disclose an interesting and sometimes puzzling appearance. The hilus shadow and heavy trunks are increased in area and density, but sharply defined, radiate, often reaching close to the periphery. These changes are usually diffuse rather than localized and there is also an absence of the interweaving and studding characteristic of the tuberculous cases.

In the probably tuberculous cases some of the characteristic alterations are seen but the picture is not complete. There may be the changes in the shadows cast by the hilus and heavy trunks and suggestive alterations in the linear markings, or some other atypical alteration. These are the cases that require the most careful study and judgment in reaching a diagnosis. The history, symptoms and physical examinations are essential in these cases.

The moderately advanced and advanced cases of pulmonary tuberculosis are readily recognized. In the less advanced cases, careful study will disclose the alterations in the linear markings, heavy trunks and hilus shadow previously described. If in such cases the fine linear markings are fuzzy, or seem to merge to form a cloud effect, such as a film of tobacco smoke in a close room, an active tuberculosis would be suggested.

On the other hand if the linear markings in a limited area are sharply defined and dense and show heavy studdings beyond the trunks, a healed lesion is suggested. This condition is emphasized if it is accompanied with heavy coarse interweavings which reach to or near the periphery. The heavy trunk leading to such an area is usually broad and dense as is the hilus on the same side. Numerous calcified areas would complete the picture, but such a diagnosis should not be made from the plates alone.

One other change which is almost constant in pulmonary tuberculosis, but which is less often diagnosed, either clinically or radiographically, is pleurisy. Marked localized thickenings and interlobular pleurisies are recognizable, but the evenly distributed pleurisy which spreads uniformly over one or both pleural surfaces usually defies detection. On the other hand, pleurisy with effusion, even of moderate magnitude, is readily detected and presents a most interesting appearance in the stereo-rœntgenograph. The fluid is seen to be pressed up around the periphery of the chest cavity. The line of contact between the shadow of the fluid and the shadow of the lung is not, however, a smooth rounded surface, but rather a series of cup-like depressions. Apparently the pressure of the lung upon the fluid is not uniform, there being bands between which the pressure upon the fluid is greater than at the bands. Emerson has compared the position of the pleural effusion pressed upon by the lung with the position of water in a bottle pressed upon by an inflated rubber bag. The simile would be more exact if the rubber bag were covered by a balloon net. Regarding the oft repeated statement that in pleurisy with

effusion the upper border of the fluid moves on change of position of the patient; the X-ray shows but slight if any change in the fluid level whether the patient be in the erect or recumbent position.

The report of Dr. Wolman clearly illustrates the value of this method of studying pulmonary lesions. So far as we know, this is the first report of any careful stereo-rœntgenographic study of the alterations in the fine linear markings occurring in pulmonary disease and the application of this knowledge to the diagnosis of pulmonary tuberculosis. We hope in the near future to be able to explain on anatomical grounds the alterations in the appearance of the linear markings. However, although the method is still empirical and controlled only by careful clinical examinations, we are firmly convinced of its great worth and feel that its more general adoption would greatly increase the value and accuracy of the radiographic diagnosis of pulmonary conditions.

## PART II.

By Walter W. Boardman, M. D., and Kennon Dunham, M. D.

There is considerable diversity of opinion as to the value of Rœntgen rays as an aid in the diagnosis of early pulmonary tuberculosis, some authorities placing them above ordinary physical examination, others on a par with physical examination and still others denying to them any such position. To investigate this matter it was decided to install an X-ray apparatus in the laboratory of the Phipps Dispensary and to study an extensive series of cases, controlled by careful clinical examination, tuberculin tests, etc.

In reading an X-ray plate, as in the study of a pathological specimen, it is first necesary to become familiar with the normal before trying to interpret the abnormal. Further, as in studying a pathological specimen it is important to know the structure and arrangement of the various units, so in an X-ray plate it is important to know the anatomical basis for the shadows seen. We therefore turned our attention to a consideration of this subject.

Remembering that in an X-ray plate we have merely a record of differences of density,—the tissues of greater density appearing as light areas, those of less density as dark areas,—we know that the shadows seen correspond to structures in the chest possessing greater density than the structures immediately surrounding them. On the single plate we have of necessity various shadows superimposed one upon the other. In stereoscopic plates, however, the chest is seen in perspective, the shadows occupying the same relative position in the image as the structures which cast these shadows occupy in the chest. The advantage of the stereo-rœntgenogram for the careful study of the various shadows must therefore be self-evident.

Turning then to a consideration of a stereo-rœntgenogram of a normal chest we see the shadow picture of the bony framework, with its covering of soft parts enclosing the chest except inferiorly, where the shadow of the diaphragm is seen. The shadows within the chest cavity seen, as we have stated, in their normal relations, may readily be divided into three main groups: first, the heavy central shadow extending from the upper boundary of the chest cavity to the diaphragm; next the hilus shadows, radiating irregularly to a distance of four or five centimeters on either side of the center of the preceding shadow, and finally the finer markings seen in the lung fields.

Owing to imperfections of apparatus, the hilus shadows and the finer markings were rarely, if ever, seen upon the plates of the earlier workers and it was not until 1900 that they attracted any serious consideration. Thus Hickey [1] states that we find various shadows constantly present which are interpreted as shadows of the bronchi by some, shadows of the pleura by others, and shadows of consolidation by still others. However, he believed them to be perfectly normal, and basing his opinion on various injections, concluded that they were the shadows of the larger pulmonary vesels.

Holzknecht [2] believed that the hilus shadows were due to pulmonary blood vessels; but to the smaller vessels situated near the surface of the chest next the plate, rather than to the large centrally situated vessels.

De la Camp [3] disproved Holzknecht's contention by X-raying serial frontal sections of a cadaver. By this means he definitely proved that the hilus shadows were due to organs which originate at the root of the lung and branch out toward the periphery and are not due to formations near the plate. From various injection experiments he also concluded that the bronchi were responsible for these shadows and that the vessels took practically no part in them.

Holzknecht and Kienböck [4] rejected the conclusions of De la Camp and stated that they believed the pulmonary vessels were primarily responsible for the shadows and that the bronchi participated in them to but a slight extent.

De la Camp,[5] after further careful experimental work, acknowledged that the blood vessels might cast some shadow, but held that they were secondary in importance to the bronchi as a source of the hilus shadows.

Cowl [6] very definitely states that the shadows normally present in the fields of the lungs are due to the ray-like spreading lung vessels and bronchi, but he brings forth no proof of his statement.

Rieder [7] speaks of the irregular spotted markings seen in the lung fields and states that they are caused more by the pulmonary vessels, especially the arteries, than by the bronchi. Later he states that the anatomical nature of the shadow stripes and hilus shadows has not yet been determined, but that most investigators believe both bronchi and blood vessels play a part in the production of these shadows.

Cunnington [8] describes a "tree branch striation" which he noted in only a few of his tuberculous cases and which he believes is due to the distended lymphatics draining the involved areas.

Many others have expressed opinions regarding the anatomical basis of these shadows, but have made no attempt to prove their statements. As Rieder has said, the most generally accepted opinion regarding these shadows is that they

are due to both blood vessels and bronchi. However, in 1910 Fraenkel and Lorey,* after some careful experimental work, conclude that "the anatomical sub-stratum of the hilus shadow consists entirely of the blood vessels of the lungs and that under normal conditions the bronchial arborization gives no shadow on the Röntgen plate. It is, however, quite possible that in abnormal conditions, dilated bronchus filled with pus, it may give a shadow."

From the foregoing references to the literature it is evident that the question as to the anatomical basis for many of the shadows seen in X-ray plates of the chest had not been settled. In our investigations upon this question the work falls into four divisions: first, studies of stereoscopic X-ray plates of normal and diseased chests before death; second, studies of stereoscopic plates taken shortly after death, the X-ray reading being carefully compared with the autopsy findings; third, studies of stereoscopic X-ray plates of animal and human beings in which blood vessels and bronchi had been injected with bismuth or other similar materials; and fourth, studies of stereoscopic X-ray plates of the lungs of animals and human beings upon which more or less dissection had been done. The use of the stereoscope and the dissection were of greatest value.

From our study of the stereoscopic X-rays of normal and diseased chests, several points were settled. First, the hilus shadow is due to structures originating at the root of the lung and radiating toward the periphery, as demonstrated by De la Camp, since with the stereoscope these shadows were seen in their normal relations to the other structures in the chest. Second we were able to divide these radiating shadows in the hilus region and also in the lung fields into groups corresponding to the anatomical division of the lung into lobes; three on the right, two on the left. These subdivisions were very beautifully seen in the stereoscopic plates of injected lungs.

The shadow cast by the heart, with its surrounding pericardium and by the aorta, is too well known to require comment. The tracheal shadow, in many plates, could be definitely traced from the larynx into the hilus shadow as a band of slight density lying between parallel bands of great density; in several cases the divisions and subdivisions could be clearly traced well out into the lung fields. Aneurisms and dilatations of the arch of the aorta were readily recognized and their location determined.

From our autopsy work we satisfied ourselves that enlarged and calcified lymph glands may be easily recognized if occurring about the edge of or outside the heavy central shadow; if they occur within the heavy central shadow it may be impossible to pick them out. Without doubt the heavy central shadow is cast by structures in the mediastinum; the heart with its pericardium and great vessels, the oesophagus, the walls of the trachea, the lymphatic glands and the mediastinal connective tissue. But it is difficult or impossible to recognize the shadows corresponding to some of these different structures.

One of our autopsy experiments gave interesting information concerning the shadow seen along the right side of the vertebral shadow and extending from the clavicle to the base of the heart. This shadow must be cast by structures on the right side of the superior mediastinum. Here we have the superior vena cava surounded by lymphatics and mediastinal tissue. In order to demonstrate what part, if any, this vessel played in the production of this shadow, a body was placed upon the table and a set of plates taken. The right internal jugular vein was then opened and a rubber tube filled with lead shot passed some distance into it and a second set of plates taken. In these the shadow of the tube and shot can be seen extending from the neck through the shadow under consideration and into the heart shadow. At autopsy the tube was found to have traversed the right internal jugular, the right innominate, the superior vena cava, the right auricle and to have entered the inferior vena cava. We therefore feel justified in stating that the superior vena cava, with its contained blood, is at least in part responsible for the shadow seen; the lymphatics and fibrous tissue, however, must play some part in its production.

The results obtained from our injection experiments of animal and human lungs were disappointing. Plates taken after injecting bismuth into the trachea and bronchi showed shadows apparently corresponding in position and size to the shadows normally seen at the hilum and in the lung fields. However, plates taken after similarly injecting the blood vessels disclosed shadows also apparently corresponding in position and size to the normal shadows. If both bronchi and blood vessels were injected the shadows appearing on the plates were too dense to allow of careful study. Hence as a means of settling the disputed question this method was valueless, as might have been inferred by the widely different conclusions reached by the earlier investigators using the method.

Our dissection work on animal lungs was more satisfactory, especially the series in which pigs' lungs were used. These gave very beautiful pictures showing the bronchi as areas of decreased density. Partial injections of pigs' lungs showed the artery to be directly in contact with one side of the bronchus, the vein in contact with the other side. An instructive experiment consisted in carefully separating the lobules from over a bronchus, which was exposed throughout its course from hilum to periphery. A section of the bronchus was now removed and placed on another portion of the lung. The plates taken showed first a definite shadow cast by the removed bronchus; second, definite shadows cast by the artery and the vein in the space from which the bronchus had been removed. These latter shadows were less dense than the shadows with which they were continuous above and below. In other words, the arteries and veins cast shadows in X-ray plates of pigs' lungs, but normally their shadows are augmented by the shadow of the bronchus. Thus the veins, the arteries and the walls of the bronchi cast shadows of great density in the pigs' lungs; the lumen of the bronchi appears as a shadow of slight density. It now became important to repeat this experiment on a human lung.

To this end a normal human lung was obtained as soon

after death as possible, inflated to its natural degree and the first set of plates taken. In these, lines of increased density were readily seen, radiating from the root of the lung toward the periphery. The lung tissue in the region corresponding to one of the more definite lines was carefully separated until a vessel was exposed throughout its course. In the second set of plates the shadow cast by the exposed vessel, a vein, was seen to correspond in position to the shadow on the first plate. The vein was now carefully removed and placed across another portion of the lung, and a third set of plates taken. In these a definite shadow was cast by the removed vein and a shadow was still present in the original position but of lessened density. Beneath the vein lay the artery and this was now removed and placed parallel to the vein, and the course of the bronchus which was now exposed was mapped out with two pins. In the fourth set of plates the bronchial shadow was distinct, although faint, running between the pins, and a definite shadow was present corresponding to the removed artery. The bronchus was now removed and placed parallel to the vein and artery. The plates now showed an absence of any shadow in the original location and definite shadows cast by the vein, artery and bronchus. The proximal end of the bronchus in which cartilage was present cast a very marked shadow. A section of the primary bronchus and of the large thick walled pulmonary artery was now placed side by side on another portion of the lung, and X-ray plates were made. The plates showed a very definite shadow cast by the primary bronchus and a much less marked shadow cast by the walls of the pulmonary artery. Finally two other vessels were exposed and by means of syringes blood, to which a small amount of sodium citrate had been added to prevent coagulation, was injected directly into them. The plates of this last experiment showed slight but definite increase in the density of the shadow beyond the tip of the syringe needles, the site of the injected blood.

We believe that we have proved in this experiment beyond the question of a doubt that the walls of the arteries, the walls of the veins and the walls of the bronchi cast shadows in X-ray plates of normal lungs removed from the body and that these structures bound together as they are by a small amount of fibrous tisue and surrounded by the lymphatics, are collectively responsible for the shadows seen in the parenchyma of the lungs. Furthermore, that the blood normally contained in the pulmonary vessels increases to some degree the density of the shadow cast, and finally, that the walls of the large bronchi are capable of casting very definite shadows which normally are heavier than the shadows cast by the walls of the large vessels. However, the large vessels, with their contained blood, cast a homogenous shadow, whereas the bronchi, with their contained air, cast a very different shadow, consisting of two parallel bands of great density separated by an area of slight density.

We therefore believe that the anatomical basis of the shadows seen in the lung fields and at the hilum in X-ray plates of normal chests is definitely settled. The hilus shadow is caused by the primary branches of the pulmonary vessels with their contained blood and by the walls of the primary branches of the bronchi, together with the lymphatic glands and fibrous tissue which accompany these structures and bind them together. Remembering that the large blood vessels cast a uniform shadow, the blood vessels probably account for a greater part of the hilus shadow than do the two parallel shadows cast by the branches of the bronchi; however, this bronchial shadow is a very definite part of the general whole. The shadows seen in the lung fields are also due to the blood vessels with their contained blood and to the walls of the bronchi. The surrounding fibrous and lymphatic tissue is normally so slight that it probably plays little part in the production of these shadows. Here again the blood vessels, with their contained blood, must cast more shadow than do the walls of the smaller bronchi with their contained air, but it is evident that this bronchial shadow cannot be neglected as a factor in the production of the finer markings in the lung fields.

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## PART III.

By Samuel Wolman, M. D.

The purpose of this paper will be better accomplished if I confess to you that in the first few days of the work, I conceived a profound distrust of diagnoses that had to be based on Dr. Dunham's peculiar stereoscopic markings. But now I can testify to my conversion to a belief in these same scorned markings. The original distrust was as natural as the consequent conversion, for the markings and interweavings declared to be pathognomonic of tuberculous changes in the lung tissue, had no assignable anatomical or even clinical basis. We had all read of tubercles large and small, of caseated areas, of foci of softening, of thickening and congestion around all such areas, even of gross tuberculous fibrosis, yet nothing of all this was competent to explain why, for example, in the presence of a small tuberculous focus at an apex, a peculiar characteristic, recognizable, and yet delicate tracery should develop throughout a large surrounding area, and extend to the very periphery of the organ; and the more advanced the lesion the more extensive, the grosser this tracery. These markings being unexplained, mysterious, not attachable to our existing system of knowledge, had then for us no meaning. Nor even now do we know of any acceptable explanation. However, after sub-

jecting these markings to a severe clinical criticism, we can state that undeniably they possess empirical truth.

The clinical notes of all the cases reported in this paper, were made before the radiographs were taken. The radiographic study and diagnosis, on the other hand, were made without any knowledge of the history or clinical findings. Needless to say, the appearance of the patients themselves was not such that snap-shot diagnoses could be made by the radiographer, for we did not send Dr. Dunham emaciated and buxom patients alternately for examination. Indeed, in order to make the test severe, we sent comparatively few advanced cases, since the plates of these showed gross markings, such as are easily seen on single plates, and it was the stereographic markings that we were most curious to study. And then too, our study of the advanced cases soon convinced us that our clinical technique could be safely subjected to a more searching criticism, for there was an extremely gratifying coincidence of the physical and radiographic findings. And here it may be well to emphasize that the stereographic plates show not less, but more, than the single plate readings, so that not only were we testing the radiograph, but the radiograph, in turn, was testing the clinical work.

In recording the clinical findings, our aim was first to note every abnormality of the physical signs; secondly, on the basis of history, symptoms, sputum examination, tuberculin tests, and physical signs, to decide whether these signs should be attributed to tuberculosis or not. The radiographer, in his turn, noted all abnormal markings in the lungs, and would then commit himself in writing as to their etiology.

This series embraces 92 cases, and in only seven of the 92 was there a disagreement between the clinical and the X-ray findings. However, in six of these seven, the disagreement was only partial and comparatively slight, so that in only one of the 92 cases was there a definite clash.

Let us first study the six instances of only partial agreement:

CASE I.—**Clinically, impairment at right apex. The stereoscope shows impairment at both apices, but much more marked at the right. Both clinical and X-ray diagnoses are: not definitely tuberculous.**

CASE II.—**Age 5. Clinically, transient râles in the upper chest on each side, which we were not willing to diagnose as tuberculous. The X-ray suggests tuberculosis in both upper lobes. The 1 per cent tuberculin eye test was positive.**

CASE III.—**A girl of 16 with mild mitral insufficiency. Clinically, fibrous changes in right upper and lower lobes, with a definite consolidation at the left base. This girl had but few symptoms, except some weakness, and a moderate cough. She was examined by Dr. W. S. Thayer. The question arose as to whether the insufficiency could explain the lung signs. The X-ray showed the changes discovered in the right chest to be of a tuberculous nature. The consolidation at the left base was not shown, but Dr. Dunham admits difficulty in reading the left base, especially where the heart is enlarged.**

CASE IV.—**Clinically diagnosed as tuberculous at the right apex. The X-ray diagnoses lesions at both apices, but more marked on the right side.**

CASE V.—**Clinical diagnosis; marked involvement of the right lung. On the left, a large area of hyperresonance with a few crackles. The X-ray agrees as to the right lung, but shows also a large lesion in the left. (This may have been concealed, however, by the compensatory emphysema noted clinically).**

CASE VI.—**Colored woman. Wasserman test +, eye test ++ (5 per cent). Clinically, only an impaired note over both upper lobes, which we were chary of diagnosing as tuberculous on account of the lues. X-ray diagnosis, tuberculosis in both upper lobes.**

CASE VII.—**The one of disagreement—concerns a child of three years. The skin-test was positive; otherwise the examination was clinically negative, but the X-ray showed a small lesion in the left lung.**

These seven cases comprise our entire list of differences, and we feel that neither the clinician nor the radiographer emerges badly wounded. Hereafter our talk is of harmony, and as follows.

The next group is of 38 cases, in which, clinically, the diagnosis of pulmonary tuberculosis was made definitely. Twelve of these are far advanced cases, and in 16 tubercle bacilli were found. In all these 38, there was agreement between the clinical findings and the plates, as to the position, grade, and general extent of the lesions. (But it must be said that in every case, the pulmonary disturbance, as judged by the interweaving, is more extensive than the lesion, as judged by clinical means. Even a slight apical lesion appears as a long track from the root). Four of the cases were clinically stated to be fibroid. The same diagnosis was made by the X-ray. However, two cases which had at one time given definite physical signs, and had now been for some time without symptoms, and almost without signs, were reported by the radiographer as tuberculous, without any saving note. And here we must state that the X-ray, even with the stereoscope, does not seem to be able to differentiate active from healed lesions. However, neither do the physical signs.

We then have 24 cases in which definite physical signs were found. For these signs a tuberculous basis was suspected, but could not be definitely affirmed, clinically. In all these 24, the X-ray showed abnormalities corresponding to the physical signs, and in 12 of the 24, a diagnosis of tuberculosis was made. It is extremely interesting to find that in 12 of these cases, that is, in 50 per cent of those marked only probably tuberculous, clinically, the radiographer too, made a diagnosis of probable, rather than positive, tuberculosis. This conservatism on the part of the stereograph, was extremely reassuring to those of us who were timid about relying on the mysterious interweavings. Case VI of this series had a mitral lesion, which made us hesitate in diagnosing tuberculosis. The X-ray said tuberculosis, and the sputum showed tubercle bacilli. However, Case VIII, which the X-ray diagnosed as only probable, we fully believe is tuberculous, on the basis of later signs and symptoms.

Case XIV is one of general bronchitis, with indefinite signs at the apices, but severe constitutional symptoms. Although clinically, we were not ready to diagnose the case as tuberculous, yet we were strongly tempted to do so. However, the

X-ray note made was "borderline case. Suggests thickened bronchi." Later events showed no ground to suspect tuberculosis.

In Case XIX, the clinical diagnosis was, thickened pleura, bilateral. The X-ray note was, "Pleurisy at the apices. Probably an old tuberculous lesion."

In Case XXII, the clinical finding was impairment at both apices, but more on the left. No râles. The X-ray reports both apices involved, but the older lesion on the left.

The next group comprises 14 cases. In these 14, neither the X-ray nor the physical examination noted any abnormalities. It should be remembered that in the entire series of 92, there was only one other case in which the chest was physically negative, and in that case, as already stated, the X-ray showed a lesion. This accord as to absolutely negative findings on both sides of the investigation in 14 out of 15 cases, so reported clinically, is of considerable significance.

The last group comprises eight cases. In these eight, the diagnosis of "not tuberculous" was made by both methods, but in contrast to the preceding group, physical signs were present, and were similarly reported by the radiographer, and by the clinician. It should here be noted that evidently the stereograph does not report all abnormalities in the pulmonary parenchyma as tuberculous. ( This indeed appeared also in the group of probable cases, as noted above). Case I of this series had definite tertiary lues elsewhere in the body. Case II was one of bronchial asthma. The X-ray reports signs in all five lobes, but they suggest bronchial trouble, rather than tuberculosis. The patient, at the time the picture was taken, did not give evidence of having secretion in the bronchi. The lungs that day seemed clear. In this case, too, pleural thickening over the right upper lobe was diagnosed by both methods. In Case III, Dr. Dunham refused to make a diagnosis, but said the plate showed what was perhaps a healed lesion. The clinical history, too, suggests an old infection, whatever the etiology.

We wish further to mention two cases. Both were young adults, both in apparently good health, but each had a history of chronic cough and expectoration. Physically, there were signs of severe general bronchitis. However, in each case the râles were more numerous on the right side, and there was moderate impairment over the upper lobe of the right lung. The stereograph diagnosed tuberculosis in both cases, more marked in the right lung. The patients, however, seemed in good health, and we might have been skeptical, had not one of them produced tubercle bacilli in the sputum. And we are inclined to believe the diagnosis in the other case, too, since the signs are more emphasized on one side.

To sum up: In only one of the 92 cases was there an entire disagreement; in only six, a partial disagreement. In 85, there was entire agreement. Of these, 39 are positive clinically, 24 are probably clinically, and 12 of these 24 are diagnosed as only probable by the X-ray. In eight cases, physical signs were found by both methods, but they were diagnosed as not tuberculous, and in 14 cases, there was an agreement as to the entire freedom of both lungs from abnormalities.

We think these figures rather convincing as to the ability of the stereograph to discover abnormalities and to diagnose them; and although in the great bulk of cases, it tells us no more than a careful clinical examination, yet in a fair number of cases, and these among the most interesting and puzzling, it gives additional information. But we must add the caution that a careful history is indispensable, since not even the stereograph can tell an active from a healed lesion.

### Discussion.

Dr. F. H. Baetjer.—I have listened with a great deal of pleasure to these three papers and must congratulate the authors on the admirable work they have presented to us tonight.

Dr. Boardman's paper was of great interest to me as I think that he has definitely settled the question as to the meaning of the linear radiation that we see in the X-ray plate of the lungs. As you have been told various theories were held as to the meaning of these lines, some looking upon them as simply being the small bronchi, others as being due to the veins and still others as being due to the arteries. I think that he has shown positively that these lines are due to all three. Dr. Dunham's paper was of special interest to me in that I do not agree with him absolutely as to the interpretation of the linear markings. According to his classification the lung is divided into three zones, first, the markings just outside of the sternum, and these he calls the hilum shadows; second, the prolongation of these shadows out a little beyond the middle of the lungs, and third, the zone that is made up of the linear markings extending to the periphery of the lungs. It is especially with the third zone that we have to deal as it is upon these markings that Dr. Dunham makes his diagnosis of early tuberculosis. These lines are not normally present and he has found that when they are present and extend to the periphery it is due to a tuberculous change. As he himself says this method of diagnosis is empirical and has no pathological basis but that his findings have also been substantiated by the clinical findings. Now it seems to me that this is the point where error could so easily creep in. In the first place the only check upon X-ray findings is the clinical findings and it seems to me that this is not an absolute proof. Secondly, it seems to me that any chronic infection of the lungs could just as easily produce changes in the bands which carry the artery, vein and bronchus. In emphysema and chronic bronchitis we see a marked thickening of these bands extending throughout the lungs. I agree with Dr. Dunham that the irregularities along these linear markings which he has termed "fuzzy" are collections of small tubercles but they are seen in a later stage of the disease. Since the X-ray will give us the slightest changes in density the X-ray plate will naturally record very fine and minute changes. In many of the plates these linear markings are very fine and it seems to me that it is impossible for the clinician either by percussion or auscultation to detect them. Our own experience here in the hospital has been that the radiograph will invariably show greater changes than the clinical findings. Consequently in these very early cases where

the clinical diagnosis agrees with the X-ray we are forced to conclude that either the radiographs do not show all the changes in the lung substance or that the auscultation and percussion have reached a degree of surprising accuracy.

Dr. Dunham deserves a great deal of credit for this piece of work but at the same time my feeling is that we must have pathological instead of clinical substantiation before we can absolutely accept this method for the determination of very early tuberculosis.

DR. L. V. HAMMAN.—Dr. Baetjer has discussed these papers from the technical standpoint and perhaps it would be well to say a few words about the early diagnosis of pulmonary tuberculosis from a clinical standpoint. During the six years that the Phipps Dispensary has been in existence we have given more study to this particular question than to any other and the change that has gradually come about in our attitude towards it has been a matter of surprise and of the keenest interest to us. When the dispensary was started those of us who worked there had the average training and skill that comes from some years of medical service in hospital and dispensary. This we considered sufficient for our needs, but from constant and continuous application to pulmonary diagnosis we soon developed a skill that made apparent abnormal physical signs that had previously been overlooked. This was the period of greatest satisfaction and confidence for the signs meant trouble in the lungs and trouble in the lungs spelt tuberculosis and pulmonary tuberculosis needed energetic treatment and such treatment was followed by improvement. We had innumerable cases of early pulmonary trouble in those days, many more than you could find in our records now. We were further intrenched in our confidence by the confirmatory evidence lent by tuberculin. To give added assurance we administered tuberculin to these patients subcutaneously. Of about 36 patients so tested at Eudowood all but one reacted. Slight physical signs and the tuberculin reaction played so smoothly into one another's hands that we at last became suspicious and began to give tuberculin indiscriminately to as many patients as we could control and to our dismay found that about 60 per cent of all individuals react, whether they are suspected of having tuberculosis or not. The advent of the local tuberculin tests allowed us to apply tuberculin on a large scale and to verify our previous observations by convincing numbers. This was the check that made us review more cautiously our previous conviction and led to a less ready and more difficult judgment of our cases. Individuals that formerly would have been peremptorily condemned were now kept under observation instead of being rushed to a sanatorium, and somewhat to our disappointment, they continued well under ordinary conditions of life in spite of the persistence of the abnormal physical signs.

This has brought us to the period where we now are in which the clinical symptoms play such an important rôle in distinguishing active from inactive lesions—of course I cannot go into details.

Some weeks ago a prominent man from the medical clinic came into the Phipps Dispensary to see a patient and casually remarked "of course the patient has tuberculosis." He was told no we really thought the man did not have tuberculosis, when with assumed surprise he replied "why I had no idea that you ever failed to find tuberculosis in a patient." This was to us a very painful display of lack of knowledge of our aims and methods. However, some days later, speaking to a physician extremely active in tuberculosis work, I mentioned that we had grown more conservative in the diagnosis of early pulmonary tuberculosis and he rebuked me with "yes, I know you have, and even too conservative."

The dispensary then is in a not very enviable position—too hot for medical men and too cold for the specialists. In the predicament the X-ray comes in and offers a helping hand.

The result of the valuable work that Drs. Dunham, Wolman and Boardman have done that interests me most, probably because it brings a ray of comforting reassurance, is the remarkable correspondence between the results of the physical examination and the plate readings. Here is at least something definite that we can hang too. A skillful physical examination—and I emphasize skillful—outlines for us with unexpected precision even slight changes in the lungs. The interpretation of these changes may be and indeed is an occasion of keen discussion. With the objective verification it can no longer be insinuated that the signs are the expression of an imagination stimulated to overwork by a prurient enthusiasm, nor that even such minor changes come within the range of normal variation.

There are three points in the X-ray work that particularly deserve emphasis.

1. The lesion in all cases is more extensive than the physical signs indicate. We have already learned this from autopsies and especially from focal tuberculin reactions and have become accustomed to visualize further than a strict interpretation of the physical signs would permit.

2. While in all of our early cases we discover the earliest signs at the apices the X-ray plates show the most marked changes at or near the hilus and bands radiating from here to the apices. This is hardly in accord with anatomical findings and I would ask Dr. Dunham if he can tell us what is the pathological basis of these dense radiating bands.

3. And most important of all I would say that interpretation of these stereoscopic plates is as expert a bit of skill as the physical examination. It is certainly no reflection upon Dr. Dunham's work when I confess that I am unable to see in the plates all that Dr. Dunham sees there. From plate readings alone Dr. Dunham will diagnose one case definitely pulmonary tuberculosis, and another as suspicious. From physical examination alone we are unable to do this. We turn back and rely on the clinical symptoms to decide whether the lesion is an active or inactive one.

DR. W. S. THAYER.—I have been considerably impressed during the last year or so with the real value of the radiograms of the chest in suspected tuberculosis.

The diagnosis of an early or later pulmonary tuberculosis

is often made only by the gathering together and the carefully weighing of a variety of special observations and the results of a radiographic examination are not infrequently of great importance in leading one to the correct conclusion.

The remarkable agreement between the results of physical examination and the radiogram in the cases reported this evening are extremely satisfactory and encouraging. They go to show how much can be made out by a true, careful physical examination.

The wonderful stereoscopic pictures which Dr. Dunham has been able to show us, mark a real step forward in our methods of thoracic investigation.

# PAROXYSMAL HEMOGLOBINURIA:
## BLOOD STUDIES IN THREE CASES.

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The studies herein reported have been directed toward the phenomena which the blood of this interesting condition presents, rather than to the clinical features of the disease.

The condition was separated as a clinical entity long ago and the symptom complex sufficiently well known to render diagnosis certain and easy.

From the first recognition of the disease, investigators have busied themselves with the search for an explanation of the remarkable manifestations of the attacks and numerous theories have been advanced to account for them which now have only an historical interest and need not be reviewed, since this already has been done adequately by Eason.[1]

Our present views on the mechanism of the blood destruction during the attacks in this disease date from the publication of Donath and Landsteiner,[2] who showed that hemolysis of the blood of hemoglobinuric patients took place in vitro if the temperature was considerably lowered and then raised.

It had already been suggested, especally by Eason, that the hemolysis in these cases was due to a complex hemolysin of amboceptor-complement nature, whose action bore some relation to temperature, but the proof of the amboceptor-complement nature of the hemolysin, as well as the explanation of the part played by cold, seems not to have been furnished before the work of Donath and Landsteiner just cited.

The fundamental facts brought out by them were as follows:

1. To show the relation of temperature to hemolysis.

Blood from hemoglobinuric patients taken in potassium oxalate solution, to prevent clotting, underwent no hemolysis if kept either at low temperature or at body temperature, but hemolysis occurred if the temperature was first lowered and then raised. The same result followed if a mixture of serum and washed corpuscles from a hemoglobinuric patient was used instead of oxalated blood.

2. To show the amboceptor-complement nature of the hemolysin.

Heating the oxalated plasma to 45° C. for 15 minutes definitely lowered its hemolytic strength, while heating it to 55° C. for 15 minutes destroyed the same.

Corpuscles chilled in oxalated plasma and then removed by centrifugalization did not undergo hemolysis on the addition of normal salt solution or of inactivated hemoglobinuric serum at thermostat temperature, but were promptly dissolved after the addition of fresh normal serum.

On the above evidence the authors convinced themselves of the amboceptor-complement nature of the hemolysin and concluded that the serum of paroxysmal hemoglobinuric patients contains a hemolytic amboceptor which requires for its union with the red blood cell a low temperature, while the union of complement and consequent solution of the cell takes place only at higher temperatures.

While it is true that they bring forth some further experimental evidence in support of this view, it is perhaps significant in the light of the findings of Hoover and Stone,[3] and of my own results to be reported presently, that Donath and Landsteiner do not mention in their first communication having made any attempts to reactivate hemoglobinuric serum heated to 56° C. by the addition of fresh normal serum.

In a later publication[4] they do report successful reactivation experiments but it is to be noted that the reactivating serum (complement) was added to the mixture of inactivated patient's serum and corpuscles before the mixture was subjected to a low temperature and that their reactivation experiments failed if the hemoglobinuric serum was inactivated by subjecting it to a higher temperature than 48° – 50° C. for 20 minutes.

Hoover and Stone made very complete studies on two cases of paroxysmal hemoglobinuria in which they confirm Donath and Landsteiner's conclusions concerning the relation of cold to hemolysis and the amboceptor-complement nature of the hemolysin, as indeed most investigators subsequent to Donath and Landsteiner have done. In opposition to the views of other workers, however, they maintain that, not only does the amboceptor, but also the complement, require a low temperature for its union, the lytic action of the complement, however, only taking place after the temperature is elevated.

Their views and the experiments upon which they are based are best given by a direct quotation from their report.

"0.5 cc. of inactivated H. S. + washed R. B. C. + 5° C. for 30 minutes + 37° C. for one hour.

Now if the normal serum be added and the mixture is placed in the thermostat for many hours, no hemolysis follows.

Inasmuch as a low temperature was required to procure fixation of the amboceptor to the red blood cell it seemed probable that reactivation experiments had hitherto failed because a low temperature was not employed in fixing the complementophilic end of the amboceptor to the haptophore of the complement. The following reactivation experiment proved successful.

0.5 cc. inactivated H. S. + washed R. B. C. + 0.25 cc. of normal serum + 5° C. for 15 minutes.

The mixture was then warmed to 37° C. and hemolysis followed immediately.

Thus it is apparent that cold is essential for the fixation of both the cytophilic and the complementophilic ends of the amboceptor."

While I have repeatedly confirmed the results of the above experiments, I differ with Hoover and Stone as to their interpretation and will return to this discussion presently.

Meyer and Emmerich[5] have made very exhaustive clinical, hematological and serological studies of four cases of paroxysmal hemoglobinuria.

Reference need not be made here to their observations on the blood pressure during the paroxysms, the leucocytic changes, the appearance of the derivatives of hemoglobin in the urine after attacks, nor to the very interesting clinical observations of these authors, since the present paper does not deal with these phases of the disease.

Meyer and Emmerich report that the blood of their patients did not always show hemolysis when subjected to a low temperature followed by high temperature, as described by Donath and Landsteiner. Thus with their first patient they obtained positive results only 13 times out of 40 tests performed at intervals during five months. Similarly the test often failed to give hemolysis with their other three patients.

The negative results are ascribed in most instances to lack of complement since they found that positive results were obtained much more frequently if normal serum (complement) was added to the mixture of patient's serum and red blood cells.

They found that the complement deficit usually occurred shortly after an attack of hemoglobinuria and ascribed it to the using up of complement during the attack; although they consider that the complement content of the blood undergoes wide variation independently of attacks of hemoglobinuria.

It is interesting that the authors found this deficit of complement did not protect the patients from attacks of hemoglobinuria, for they observed that at a time when the serum could be shown to contain no complement the patient might undergo a spontaneous or induced attack; this paradoxical result they explain on the assumption that complement is formed locally in the surface of the body exposed to the action of cold.

With reference to the union of the red blood cell and amboceptor, Meyer and Emmerich state that it makes no difference whether the complement is added to the mixture of red blood cells and inactivated hemoglobinuric serum before or after cooling the mixture, hemolysis taking place equally well in either case.

Especial attention is directed to this point since it stands in opposition to the results of Hoover and Stone and myself.

Meyer and Emmerich, in summing up the results of their experiments, report that "the amboceptor becomes bound to the blood corpuscle in the cold, but is easily dissociated from the corpuscle at high temperatures." They believe that some of the contradictory reports in the literature are to be explained on this basis. Referring to the work of Hoover and Stone, they say, "While we have seen above, that the binding of the amboceptors to the corpuscles follows in the cold without the presence of complement Hoover and Stone report that the mixture must be cooled in the presence of complement. This appearance is readily explained by the easy dissociability of the amboceptor."

Hoover and Stone do not make the point that the union of red blood cell and amboceptor can take place only at low temperatures in the presence of complement, but that a low temperature is necessary for the union of complement to the corpuscle-amboceptor combination.

There is one paragraph in Hoover and Stone's report which seems to justify the meaning which Meyer and Emmerich ascribe to them, but their further experiments indicate that they did not believe that the presence of complement was necessary for the union between corpuscle and amboceptor to take place, as evidenced by the following quotation from their paper:

"Thus we have shown that if in two stages, 10 drops of a 10 per cent suspension of red cells be added to (1 cc.) the serum and exposed to cold for one hour each time, the amboceptor can be exhausted from the inactivated hemoglobinuric serum."

Furthermore, Hoover and Stone in a personal communication to me stated that they did not hold that the presence of complement was necessary in order that union take place between corpuscle and amboceptor.

With reference to the resistance of the red blood cells of hemoglobinuric patients, Meyer and Emmerich carried out experiments to show that the resistance to certain destructive agents, such as saponin, was greater if the corpuscles had anchored amboceptor than if they were amboceptor-free and therefore they conclude that it is necessary to use amboceptor-free corpuscles in resistance tests. They state that corpuscles from hemoglobinuric patients are less resistant to temperature changes, mechanical influences, dilute acids, and saponin solutions, but that they do not differ from normal corpuscles in their resistance to anisotonic salt solutions.

No attempt has been made to give a complete review of the literature on paroxysmal hemoglobinuria, or even to bring out all the facts which have been established concerning it. Reference has been confined largely to those phases of the subject with which my own work has dealt.

### CASE REPORTS.*

CASE I.—G. C., female, colored, aged eight years.

*First Admission* to hospital, February 9, 1910.

*Diagnosis.*—Paroxysmal Hemoglobinuria, Congenital Syphilis.

*Complaint.*—Blood in urine.

*Family History.*—Father and mother natives of British Guiana, South America. Father alive and well. Mother alive, subject to

* For the privilege of studying and reporting Cases I and II, I am indebted to Dr. S. Amberg, who kindly referred them to me

asthmatic attacks, menstrual periods characterized by profuse sanguinous discharge and each child-birth has been accompanied by troublesome hemorrhage.

The first child, a male, was born in British Guiana 14 years ago, and died at the age of nine years of diphtheria, followed by pneumonia. This child was subject to epistaxis, but withstood exposure to cold well.

Second child, female, died a few days after birth of umbilical hemorrhage.

The third child, female, died at the age of one month from umbilical hemorrhage. Bleeding from umbilicus began 12 hours after birth and recurred twice during the month the child lived.

Fourth child (G. C., the present patient), was born eight years ago in Washington, D. C.

Fifth child, male, alive and well.

Sixth child, still-born.

*Past History.*—Patient suffered from a vaginal hemorrhage shortly after birth. Measles at three years, chicken-pox and diphtheria at four years, typhoid fever at five years, whooping cough at six years. Has had several attacks of tonsillitis, the last being in November, 1908.

*Present Illness.*—Began five years ago, when it was noticed that exposure to severe cold would be followed in a few hours by a chill and later by fever and the passage of "bloody urine." The elimination of "blood" in the urine has never persisted more than 24 hours after the onset of an attack. Attacks have never occurred during warm weather; they have been most frequent in the spring and fall of the year, especially in cold, damp weather. The patient's mother states that prior to the onset of the present illness, five years ago, exposure to cold frequently caused a profuse eruption of hives over the face and body, but that since the onset of the attacks of hemoglobinuria the patient has never suffered from hives.

The patient suffered an attack on the day before admission to the hospital and another on the morning of admission.

*Physical Examination.*—A well nourished child, rather small for her age. Pupils widely dilated, react normally. Breathes through mouth, tonsils greatly hypertrophied, rather pale and contain deep crypts. Teeth normal. Considerable exophthalmos, von Graefe's sign positive, thyroid not enlarged.

*Lungs.*—Clear on percussion and auscultation.

*Heart.*—Not enlarged, sounds clear except for a slight systolic murmur at apex, which is transmitted but a short distance into axilla.

*Liver.*—Somewhat enlarged and readily palpable 2 cm. below costal margin in mamillary line. Spleen is hard and is just palpable at costal margin.

Patellar reflex exaggerated, plantar reflex normal, no ankle clonus.

Cervical, axillary, inguinal and epitrochlear lymph glands markedly enlarged.

Urine on admission was of a dark red, smoky color. Sp. Gr. 1015, faintly acid, contained no sugar, much albumin, and gave a positive guiac test for blood. Microscopically a few white blood cells were seen, but no red cells or casts. Subsequent examinations of the urine during the patient's stay in the hospital revealed no abnormality. The temperature on admission was 99.8° F., but fell to normal within a few hours and remained normal.

*Blood Examination.*—Fresh specimen showed no poikilocytosis or anisocytosis, no parasites seen.

| | |
|---|---|
| R. B. C. | 4,000,000 |
| W. B. C. | 22,000 |
| Hb. | 70% |

Wassermann reaction positive.

Patient discharged February 23, 1910.

Second admission to hospital, November 10, 1910.

Patient passed through the summer months without attacks, but with the onset of cold weather in the autumn the attacks began to recur.

Wassermann reaction still positive.

Differential blood count on admission:

| | |
|---|---|
| Polymorphonuclear neutrophiles | 50% |
| Polymorphonuclear eosinophiles | 10% |
| Basophiles | ½% |
| Large mononuclears | 9% |
| Small mononuclears | 30% |
| Transitionals | ½% |

The above count was made during the interval between attacks, a considerable time having elapsed since the last attack.

*November 15, 1910.*—Intravenous injection of 0.3 gram Ehrlich's "606." The patient remained in hospital until December 8, 1910. During most of this time she was kept in bed and not exposed to a sufficient degree of cold to induce an attack of hemoglobinuria. Nevertheless, on December 3, without premonitory symptoms, she voided a dark brown urine, which gave a positive guiac test for blood. Wassermann reaction positive.

CASE II.—F. L., male, colored, aged seven years.

Admitted to hospital November 6, 1910.

*Diagnosis.*—Paroxysmal Hemoglobinuria, Congenital Syphilis.

*Complaint.*—Blood in urine.

*Family History.*—Father alive and well, mother alive and well. One brother died at two months of age, cause unknown. Mother has had four miscarriages. No positive history of syphilis in either parent.

*Past History.*—At the age of one month the patient had a conjunctivitis lasting a week. At two and a half months of age, pleurisy, otorrhœa at 18 months of age, pneumonia at one year. Since then has had measles and whooping cough.

Patient was shocked by lightning 18 months ago; since then he has had at times some pain in right leg and occasional swelling of right knee and ankle joints.

*Present Illness.*—Began in April, 1908, three days before the onset of an attack of measles. After exposure to severe cold patient had a chill, folowed by fever and the passage of "bloody urine." Since that time the patient has had very numerous attacks, as many as three or four in a week. The attacks occur only in cold weather, never in the summer.

*Physical Examination.*—Normal looking child. Pupils equal and react normally. Teeth appear normal, tonsils enlarged and injected. Lungs and heart normal. Liver and spleen not enlarged. Patellar reflex normal.

*Blood Examination.*—Fresh specimen showed no poikitocytosis or anisocytosis, no parasites seen.

| | |
|---|---|
| R. B. C. | 5,488,000 |
| W. B. C. | 10,280 |
| Hb. | 74% |

*Urine Examination.*—Specimen is clear yellow. Sp. Gr. 1020. Reaction acid, no sugar, no albumen, no sediment. Microscopically no abnormal constituents seen. Guiac test for blood negative.

Wassermann reaction positive.

CASE III.—J. S., male, white, aged 32, Austrian.

*Complaint.*—Passage of bloody urine following exposure to cold.

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from the Pediatric Dispensary of the Johns Hopkins Hospital, and to Dr. L. F. Barker, who subsequently admitted the cases to his service in the Johns Hopkins Hospital and permitted these studies to be carried out.

For the privilege of studying Case III, I am indebted to Drs. Hoover and Stone, this being one of the two cases reported by them.

*Family History.*—Father died at age of 65 of "liver trouble." Mother alive, aged 65 years, has chronic rheumatism. One brother alive and well, one brother dead as result of accident, one half-brother and two half-sisters alive and well.

*Past History.*—General health has been good. As a child had scarlet fever, measles and chicken-pox. Has had grippe repeatedly and "catches cold" easily. While in the army ten years ago had an attack of arthritis, which kept patient in bed for two weeks, convalescence occupied several weeks. Pain and swelling started first in knee joint, then the hip, shoulder, wrist and temporo-maxillary joints were successively involved. Venereal infection denied.

*Present Illness.*—Began December 7, 1902. Following exposure to severe cold patient had a chill lasting one-half to one hour, succeeding which there was high fever for two or three hours. The first urine voided following the chill was "bloody." The attack was over in five or six hours. Within a week he had a second and ever since has been subject to attacks whenever he gets very much chilled. They are most frequent during spring and fall, especially during cold damp weather. He never suffers from them during the summer.

Wassermann reaction positive.

The hematological studies carried out on the foregoing cases were directed along the following lines:

1. The demonstration of the peculiar hemolysin characteristic of paroxysmal hemoglobinuria.

2. The determination of the group (according to the iso-agglutination reaction) to which the patients belong and presence or absence of normal iso-hemolysins.

3. Determination of the relation of temperature to the union of (a) amboceptor and corpuscle, (b) complement and amboceptor-corpuscle.

4. Separation of the auto- and iso-hemolysin contained in the same serum, in order to determine if the auto-hemolysin is capable of dissolving those individuals' corpuscles for which the serum in question contains an iso-hemolysin.

5. Resistance of the red blood cells.

6. Wassermann reaction.

7. Effect of the administration of Ehrlich's "606," (a) on the Wassermann reaction, and (b) on the clinical course of the disease.

For all of these studies blood was obtained from an arm vein by means of an aspirating syringe. The syringe was always washed out with sterile sodium citrate solution before using in order to remove the small amount of water which remains in the syringe after boiling and which might cause slight laking of the blood.

Having drawn the blood into the syringe, 2 or 3 cc. are introduced into a centrifuge tube containging 12 cc. of a 1.5% sodium citrate in 0.85% sodium chloride solution for corpuscles. The remainder of the blood is placed in a sterile centrifuge tube and allowed to coagulate at 37° C. in order to obtain serum. After the clot has formed the separation of the serum is facilitated by a few minutes' centrifugalization. The clear serum, which must be perfectly free from hemoglobin, is now pipetted off and transferred to another tube. The further preparation of the corpuscles consists in washing them three times in 0.85% sodium chloride solution to free them from serum and in preparing a 5% suspension of the washed corpuscles in 0.85% sodium chloride solution.

1. *The demonstration of the peculiar hemolysin characteristic of paroxysmal hemoglobinuria.*

The test employed for this demonstration was carried out as follows * and always gave the same result:

Pt. serum 0.25 cc. + Pt. corp. 0.25 cc. (5 % susp.) 0° C. ½ hr. 37° C. 2hrs. = + hemolysis.

Control: Pt. serum 0.25 cc. + Pt. corps. 0.25 cc. (5% susp.) 37° C. 2 hrs. = 0 hemolysis.

At no time did the blood of any of the three cases reported fail to give hemolysis when subjected to the cold-warm test as indicated above, notwithstanding the fact that repeated tests were made, especially in Case I, which has been under observation for over a year. This is in rather striking contrast with the infrequency of positive results reported by Meyer and Emmerich (only 13 times positive in 40 tests) but may possibly be explained by the fact that I made no especial effort to examine serum from my cases at short intervals after attacks.

2. *The determination of the group (according to the iso-agglutination reaction) to which the patients belong, and presence or absence of normal iso-hemolysins.*

It has been shown[6, 7] that all individuals, regardless of health or disease, can be divided into four groups, according to the ability of their serum to agglutinate the corpuscles of other individuals and of their corpuscles to be agglutinated by the serum of other individuals.

This classification may be stated as follows:

Group I. Serum agglutinates no corpuscles. Corpuscles agglutinated by the serum of Groups II, III and IV.

Group II. Serum agglutinates corpuscles of Groups I and III. Corpuscles agglutinated by the serum of Groups III and IV.

Group III. Serum agglutinates corpuscles of Groups I and II Corpuscles agglutinated by serum of Groups II and IV.

Group IV. Serum agglutinates corpuscles of Groups I, II and III. Corpuscles agglutinated by no serum.

The group to which an individual belongs is established shortly after birth and thereafter undergoes no change.[8] A strict classification of individuals according to the iso-hemolytic properties of the blood has not been accomplished since this reaction, unlike the iso-agglutination reaction, is not entirely constant; certain general laws, however, have been formulated as a result of observations on the iso-hemolytic reaction. Thus the serum of Group I, which contains no iso-agglutinin, never contains an iso-hemolysin, while the corpuscles of Group I may or may not in a given case be hemolysed by the serum of a member of Group II, III or IV.

The serum of a given member of Group II may or may not contain an iso-hemolysin, but if it does it can act only against corpuscles of members of Groups I and III, but not necessarily against the corpuscles of all members of these two

* Where the expression "0° C." is used in this paper in describing my own experiments it is to be understood that the tubes were placed in a vessel of ice water ranging in temperature from 0° C. to about 2° C.

groups. Similarly, if a member of Group III contains an iso-hemolysin it can act only against the corpuscles of Groups I and II. The iso-hemolysin of Group IV is effective only against corpuscles of Groups I, II and III. The corpuscles of Group IV are not subject to the action of iso-hemolysin and from what has just been said, the corpuscles of members of the other three groups are never hemolysed by the serum of a member of the corresponding group.

The group to which an individual belongs can be readily determined by testing the agglutinating action of his serum against the corpuscles of the four different groups, or by testing the action of serum from the four different groups against the corpuscles of the individual to be classified. This will be made clearer by taking an example.

To determine the group to which an individual, X, belongs:

| | First Method. | 37°C. 2 hrs. | I. | II. | III. | IV. |
|---|---|---|---|---|---|---|
| 1 | Serum X 0.25 cc. + Corp. Gr. I 0.25 cc. | | 0 | + | + | + |
| 2 | Serum X 0.25 cc. + Corp. Gr. II 0.25 cc. | | 0 | 0 | + | + |
| 3 | Serum X 0.25 cc. + Corp. Gr. III 0.25 cc. | | 0 | + | 0 | + |
| 4 | Serum X 0.25 cc. + Corp. Gr. IV 0.25 cc. | | 0 | 0 | 0 | 0 |

Column I shows the resulting agglutination if X is a member of Group I and similarly, Columns II, III, and IV show the resulting agglutination if X is a member of Group II, III or IV, respectively.

| | Second Method. | 37°C. 2 hrs. | I. | II. | III. | IV. |
|---|---|---|---|---|---|---|
| 1 | Corp. X 0.25 cc. + Serum Gr. I 0.25 cc. | | 0 | 0 | 0 | 0 |
| 2 | Corp. X 0.25 cc. + Serum Gr. II 0.25 cc. | | + | 0 | + | 0 |
| 3 | Corp. X 0.25 cc. + Serum Gr. III 0.25 cc. | | + | + | 0 | 0 |
| 4 | Corp. X 0.25 cc. + Serum Gr. IV 0.25 cc. | | + | + | + | 0 |

Columns I, II, III and IV show the resulting agglutination if X is a member of Group I, II, III or IV, respectively.

An analysis of the above tables shows that in order to classify an individual it is not necessary to test his blood against blood from all four groups. It is sufficient if his serum is tested against the corpuscles of Groups II and III, or if his corpuscles are tested against the serum of Groups II and III.

Thus if the serum of the individual to be classified agglutinates corpuscles of neither Group II nor III, it must belong to Group I. If it agglutinates corpuscles of Group III, and not those of Group II, it must belong to Group II. If it agglutinates corpuscles of Group II and not those of Group III, it must belong to Group III, while if it agglutinates corpuscles of both Groups II and III, it must belong to Group IV.

It is just as easy to determine the group if one tests the corpuscles of the individual in question against the serum of Groups II and III.

By the methods just described it was determined to which groups the cases here reported belonged and the presence or absence of normal iso-hemolysin repeatedly observed. It will be sufficient to give in detail the results of a single such determination.

Serum and corpuscles were obtained, as previously described, from four normal individuals representing Groups I, II, III and IV, also from Case I, G. C., whose group was to be determined. Tests were carried out acording to the following protocol:

PROTOCOL I.—Case I, G. C.

| Tube No. | Serum 0.25 cc. | Corps. (5% susp.) 0.25 cc. | | Aggl. | Hem. | | Aggl. | Hem. |
|---|---|---|---|---|---|---|---|---|
| 1 | Gr. I. | Gr. I. | 37°C. 2 hrs. | 0 | 0 | 37°C. 2 hrs. | 0 | 0 |
| 2 | " | Gr. II. | | 0 | 0 | | 0 | 0 |
| 3 | " | Gr. III. | | 0 | 0 | | 0 | 0 |
| 4 | " | Gr. IV. | | 0 | 0 | | 0 | 0 |
| 5 | " | G. C. | | 0 | 0 | | 0 | 0 |
| 6 | Gr. II. | Gr. I. | | + | 0 | | + | 0 |
| 7 | " | Gr. II. | | 0 | 0 | | 0 | 0 |
| 8 | " | Gr. III. | | + | 0 | | + | 0 |
| 9 | " | Gr. IV. | | 0 | 0 | | 0 | 0 |
| 10 | " | G. C. | | 0 | 0 | | 0 | 0 |
| 11 | Gr. III. | Gr. I. | | + | 0 | | + | 0 |
| 12 | " | Gr. II. | | + | 0 | | + | 0 |
| 13 | " | Gr. III. | | 0 | 0 | | 0 | 0 |
| 14 | " | Gr. IV. | | 0 | 0 | | 0 | 0 |
| 15 | " | G. C. | | 0 | 0 | | 0 | 0 |
| 16 | Gr. IV. | Gr. I. | 0°C. ½ hr. | + | + | 0°C. ½ hr. | + | + |
| 17 | " | Gr. II. | | + | + | | + | + |
| 18 | " | Gr. III. | | + | 0 | | + | 0 |
| 19 | " | Gr. IV. | | 0 | 0 | | 0 | 0 |
| 20 | " | G. C. | | 0 | 0 | | 0 | 0 |
| 21 | G. C. | Gr. I. | | + | + | | + | + |
| 22 | " | Gr. II. | | + | + | | + | + |
| 23 | " | Gr. III. | | + | + | | + | + |
| 24 | " | Gr. IV. | | 0 | 0 | | 0 | + |
| 25 | " | G. C. | | 0 | 0 | | 0 | + |

This experiment shows that the corpuscles of the patient, G. C., are neither agglutinated nor hemolysed by the serum of Groups I, II, III or IV, therefore the patient is a member of Group IV. This is confirmed by the fact that her serum agglutinates the corpuscles of Groups I, II and III. It is further evident that the patient's serum contains normal iso-hemolysin, since it is able to hemolyse the corpuscles of Groups I, II and III at 37° C. without the mixtures having been previously exposed to the cold.

In addition to the normal iso-agglutinin and iso-hemolysin contained in the patient's serum, which is effective against corpuscles of Groups I, II and III without the action of cold (Tubes 21, 22 and 23) there is another hemolysin present peculiar to paroxysmal hemoglobinuria, which only becomes evident after the mixture of serum and corpuscles is subjected to a low temperature followed by a high temperature (Tubes 24 and 25).

Attention is here directed to another point of difference between the action of the hemolysin peculiar to paroxysmal hemoglobinuria and normal iso-hemolysin. I have pointed out elsewhere that normal iso-hemolysis is always accompanied or preceded by iso-agglutination. The above experiment shows that the hemolysis caused by the hemolysin peculiar to paroxysmal hemoglobinuria may occur entirely independently of agglutination (Tubes 24 and 25).

The hemolysin which characterizes the disease with which we are dealing is often referred to as an auto-hemolysin, since the patient's serum is able to bring about the solution of his own corpuscles, but as previous investigators have shown and I have repeatedly confirmed, the patient's serum is able to hemolyse not only his own corpuscles but those of other hemoglobinuric patients and normal individuals, therefore it has an iso-hemolytic, as well as an auto-hemolytic action.

This iso-hemolytic action of the hemolysin peculiar to paroxysmal hemoglobinuria, which becomes evident only by

the cold-warm experiment, is not to be confused with the normal iso-hemolytic activity which may be possessed by the serum of hemoglobinuric patients, as well as normal individuals and which does not require previous chilling for its demonstration.

Bearing in mind the fact that it does not fully define the hemolytic power of the serum, I shall use the term auto-hemolysin to distinguish that property which characterizes the serum of hemoglobinuric patients from the normal iso-hemolytic property which may be possessed by the serum of both normal and hemoglobinuric individuals.

Similar tests carried out on the other two cases showed that Case II, F. L., is also a member of Group IV, while Case III, J. S., is a member of Group III and that the serum of both cases contained, in addition to their auto-hemolysin, normal iso-hemolysin.

It now became of interest to determine if the auto-hemolysin of hemoglobinuric patients could bring about the solution of the corpuscles of all individuals; for example, corpuscles for which the serum in question contained a normal iso-hemolysin.

Before this question can be discussed, certain facts must be given which resulted from the experiments undertaken to solve the next point in the investigation, namely:

3. *Determination of the relation of temperature to the union of (a) amboceptor and corpuscle, (b) complement and amboceptor-corpuscle.*

Divergent views are held on this subject. As previously mentioned, Donath and Landsteiner hold that the union of auto-amboceptor to the corpuscle takes place only at a low temperature, that the union of complement occurs subsequently, and only at a higher temperature. Meyer and Emmerich concur in this view, while Hoover and Stone maintain that a low temperature is necessary, not only for the union of the amboceptor and corpuscle but also for the union of the complement, a higher temperature being necessary, however, for the lytic action of the complement.

It seemed easy to approach this problem by means of ordinary reactivation experiments; accordingly, some of the hemoglobinuric serum was inactivated by heating to 56° C. for 20 minutes and the following test performed: 0.5 cc. Pt. serum ina. + 0.25 cc. Pt. corps. (5% susp.) 0° C. ½ hr. 37° C. 5 min. + 0.25 cc. complement (non-lytic serum of normal individual) 37° C. 2 hrs. Result: no hemolysis. The mixture was then held at 0° C. for ½ hour, followed by 37° C. 2 hours. Result: hemolysis.

The above experiment would seem to indicate that a low temperature was necessary for the union of complement as well as amboceptor; on the other hand, it might be argued that the amboceptor united with the corpuscle during the first exposure to a low temperature, but that dissociation took place when the temperature was elevated before the complement was added, hence the failure of hemolysis at the end of the first exposure of 2 hours at 37° C. The positive result (hemolysis) occurring after the exposure to 0° C., followed by a second exposure of 2 hours at 37° C., might be explained on the assumption that reunion took place between the corpuscle and amboceptor at the low temperature and that complement now being present united as soon as the temperature reached a suitable degree and before dissociation could take place between the amboceptor and corpuscle.

That this explanation is correct seems improbable. Dissociation is said to be hastened by temperatures approaching that of the body and by mechanical measures, such as shaking. In the above experiment mechanical factors were carefully avoided and the exposure to 37° C. was very brief, only five minutes being allowed for the temperature of the mixture to rise from 0°C. to 37° C.

In the next experiment the possibility of dissociation under the influence of temperature was avoided, but mechanical factors (washing the corpuscles) were introduced.

0.5 cc. Pt. serum ina. + 0.25 cc. Pt. corps (5% susp.) 0° C. ½ hour. The corpuscles were then removed by centrifugalization at a low temperature (0° C. to 5° C.) and washed twice with salt solution at 0° C., resuspended in 0.5 cc. salt solution and 0.25 cc. of complement at 0° C. (non-lytic serum of a normal individual) added, while the suspension of corpuscles was still at 0° C. This mixture was then held at 37° C. for 2 hours, and as no hemolysis resulted the temperature was lowered to 0° C. for ½ hour and then again held at 37° C. for 2 hours. Result: no hemolysis.

The failure of hemolysis in this experiment might possibly be ascribed to dissociation of the amboceptor and corpuscle under the influence of the mechanical disturbance of washing.

The following experiment indicates that the explantions thus far offered are not correct. 1 cc. Pt. serum + 0.1 cc. Pt. corps., containing as little salt solution as possible, were subjected to a temperature of 0° C. for ½ hour, then centrifugalized at a low temperature (0° — 5° C.), the supernatent fluid removed and saved for further tests, and the corpuscles washed three times with salt solution at 0° C. to remove all the serum from them. They were then suspended in 0.75 cc. salt solution and kept at 37° C. for 2 hours. Result: no hemolysis. Complement was now added (0.25 cc. of normal non-lytic serum) and the temperature maintained at 37° C. Result: prompt hemolysis.

The following seems to be the only explanation which will harmonize the results of all the foregoing experiments: *Union between the auto-amboceptor and the corpuscle takes place only at a low temperature in the presence of complement; the complement does not enter into the combination, or at least not permanently, at this temperature. Complement does unite with the corpuscle-amboceptor combination at 37° C. with resulting solution of the cell.*

The last experiment further shows that the union between amboceptor and corpuscle is a fairly stable one, since repeated washing at a low temperature and subsequent standing for 2 hours at 37 C. does not cause dissociation.

If the above explanation is correct, then the serum in the last experiment, which was removed after standing in contact with the corpuscles at a low temperature, should have been deprived of its auto-amboceptor, but not of its complement

(provided the proper quantitative relations were observed). The following tests were made to determine these points:

0.25 cc. serum from previous test + Pt. corps. 0.25 cc. (5% susp.) 0° C. ½ hr. 37° C. 2 hrs. = no hemolysis.

0.25 cc. serum from previous test + 0.25 cc. complement (normal non-lytic serum) + Pt. corps. 0.25 cc. (5% susp.) 0° C. ½ hr. 37° C. 2 hrs. = 0 hemolysis.

0.25 cc. serum from above + 0.25 cc. Pt. serum ina. + 0.25 cc. Pt. corps. 0°C. ½ hr. 37° C. 2 hours = + hemolysis.

These three tests show that all of the auto-amboceptor had been removed from the patient's serum by contact with patient's corpuscles at a low temperature, but that complement remained, thus corroborating the findings when the corpuscles were examined and found to have anchored amboceptor, but not complement, at the low temperature.

If this interpretation of the results is correct, it furnishes an example of a hitherto undescribed function of complement; namely, the ability to effect a combination between two other bodies (amboceptor and corpuscle) without itself entering permanently into the combination.

We are now in a position to consider the question previously mentioned as to the ability of the auto-hemolysin contained in the serum of a hemoglobinuric patient to bring about the solution of corpuscles for which the same serum contains an iso-hemolysin.

4. *Separation of the auto- and iso-hemolysin contained in the same serum in order to determine if the auto-hemolysin is capable of dissolving those individuals' corpuscles for which the serum in question contains an iso-hemolysin.*

Reference to Protocol I, of experiments carried out on the serum of Case I, G. C., shows that the serum of this patient contained an iso-hemolysin for the corpuscles of a normal individual belonging to Group I, for one belonging to Group II and for one belonging to Group III (Tubes 21, 22 and 23) in addition to the auto-hemolysin for an individual belonging to Group IV and for her own corpuscles.

In order to determine if the auto-hemolysin is capable of dissolving the corpuscles of the individuals belonging to Groups I, II and III it is first necessary to remove the iso-hemolysin. To this end, serum and corpuscles were obtained as follows:

No. 3 normal individual belonging to Group III.

No. 4 normal individual belonging to Group IV.

No. 5, Case I, G. C., hemoglobinuria patient, Group IV.

A portion of serum 5 (Case I, G. C.) was inactivated by heating at 56° C. for 20 minutes and the experiments were performed as shown in Protocol II.

Tubes 1 to 9 inclusive, in Protocol II are controls and show as follows:

Tube 1 shows that serum 4, which was used as complement contained no iso-hemolysin for corpuscles 3.

Tube 2 shows that serum 5 contained an iso-hemolysin for corpuscles 3 (reading at end of first two hours at 37° C.).

Tubes 3 and 4 show that serum 5 contains no iso-hemolysin for corpuscles 4 and 5 (reading at end of first two hours at 37° C.), but that it does contain an auto-hemolysin for these corpuscles (reading after ½ hour at 0° C. + 2 hours at 37° C.).

Tube 5 shows that the iso-hemolysin in serum 5 was inactivated for corpuscles 3 by heating to 56° C. for 20 minutes.

Tube 6 shows that the auto-hemolysin (if effective against corpuscles 3) had been inactivated by heating to 56° C. for 20 minutes.

Tube 7 shows that the auto-hemolysin contained in serum 5 for corpuscles 5 had been inactivated by heating to 56° C. for 20 minutes.

Tube 8 shows that serum 4 was capable of activating the iso-amboceptor contained in serum 5 for corpuscles 3.

Tube 9 shows that serum 4 was capable of activating the auto-amboceptor contained in serum 5 for corpuscles 5.

PROTOCOL II.

To absorb the iso-hemolysin from hemoglobinuric serum leaving the auto-hemolysin and determine if the latter is capable of dissolving an individual's corpuscles (No. 3) for which the hemoglobinuric serum contains an iso-hemolysin.

| | | | | |
|---|---|---|---|---|
| 1. Ser. 4, ¼ cc. + Corps. 3, ¼ cc. | | | | 37°C. 2 hrs. = 0 hem. |
| 2. Ser. 5, ¼ cc. + Corps. 3, ¼ cc. | 37°C. 2 hrs. = + hem. | 0°C. ½ hr. | 37°C. 2 hrs. | = + hem. |
| 3. Ser. 5, ¼ cc. + Corps. 4, ¼ cc. | 37°C. 2 hrs. = 0 hem. | 0°C. ½ hr. | 37°C. 2 hrs. | = + hem. |
| 4. Ser. 5, ¼ cc. + Corps. 5, ¼ cc. | 37°C. 2 hrs. = 0 hem. | 0°C. ½ hr. | 37°C. 2 hrs. | = + hem. |
| 5. Ser. 5 ina., ¼ cc. + Corps. 3, ¼ cc. | | | | 37°C. 2 hrs. = 0 hem. |
| 6. Ser. 5 ina., ¼ cc. + Corps. 3, ¼ cc. | | | | 0°C. ½ hr. 37°C. 2 hrs. = 0 hem. |
| 7. Ser. 5 ina., ¼ cc. + Corps. 5, ¼ cc. | | | | 0°C. ½ hr. 37°C. 2 hrs. = 0 hem. |
| 8. Ser. 5 ina., ¼ cc. + Corps. 3, ¼ cc. + Comp. (Ser. 4) ¼ cc. | | | | 37°C. 2 hrs. = + hem. |
| 9. Ser. 5 ina., ¼ cc. + Corps. 5, ¼ cc. + Comp. (Ser. 4) ¼ cc. | | | | 0°C. ½ hr. 37°C. 2 hrs. = + hem. |
| 10. Ser. 5 ina., 1½ cc. + Corps. 3, $\frac{1}{16}$ cc. 37°C. 2 hrs. Centrifugalize, save serum, wash corps. | | | | |
| 11. Ser. tube 10, ¼ cc. + Corps. 3, ¼ cc. + Comp. (Ser. 4) ¼ cc. | | | | 37°C. 2 hrs. = 0 hem. |
| 12. Ser. tube 10, ¼ cc. + Corps. 5, ¼ cc. + Comp. (Ser. 4) ¼ cc. | | | | 0°C. ½ hr. 37°C. 2 hrs. = + hem. |
| 13. Ser. tube 10, ¼ cc. + Corps. 3, ¼ cc. + Comp. (Ser. 4) ¼ cc. | | | | 0°C. ½ hr. 37°C. 2 hrs. = + hem. |
| 14. Washed corps. tube 10 + NaCl ¾ cc. + Comp. (Ser. 4) ¼ cc. | | | | 37°C. 2 hrs. = + hem. |
| 15. Ser. 5 ina., 1½ cc. + Corps. 3, $\frac{1}{16}$ cc. 0°C. 2 hrs. Centrifugalize, save serum, wash corps. | | | | |
| 16. Ser. tube 15, ¼ cc. + Corps. 3, ¼ cc. + Comp. (Ser. 4) ¼ cc. | | | | 37°C. 2 hrs. = 0 hem. |
| 17. Ser. tube 15, ¼ cc. + Corps. 5, ¼ cc. + Comp. (Ser. 4) ¼ cc. | | | | 0°C. ½ hr. 37°C. 2 hrs. = + hem. |
| 18. Ser. tube 15, ¼ cc. + Corps. 3, ¼ cc. + Comp. (Ser. 4) ¼ cc. | | | | 0°C. ½ hr. 37°C. 2 hrs. = + hem. |
| 19. Washed corps. tube 15 + NaCl ¾cc. + Comp. (Ser. 4) ¼ cc. | | | | 37°C 2 hrs. = + hem. |

Wherever inactive serum was used in the above experiment it is indicated by the abbreviation "ina." Elsewhere active serum was used.

The corpuscles were used in 5% suspension except in tubes 10 and 15, where all the supernatant salt solution after centrifugalization was removed from the corpuscles before they were used.

Tube 10 was to absorb the iso-amboceptor from serum 5 inactive at 37° C. by means of corpuscles 3.

Tube 11 shows that all of the iso-amboceptor for corpuscles 3 had been absorbed from serum 5 inactive in tube 10.

Tube 12 shows that the auto-amboceptor had not been removed from serum 5 inactive in tube 10.

Tube 13 shows that the auto-amboceptor contained in serum 5 inactive was capable of dissolving an individual's corpuscles (corpuscles 3) for which the hemoglobinuric serum contained an iso-hemolysin, Q. E. D.

Tube 14 shows that the corpuscles in tube 10 had anchored an iso-amboceptor from serum 5 inactive, which was not separated from the cells by washing twice at room temperature with 10 cc. salt solution at each washing.

Tube 15 was to absorb the iso-amboceptor from serum 5 inactive at 0° C. by means of corpuscles 3. (I have previously

shown that the auto-amboceptor cannot be absorbed even at 0° C. except in the presence of complement. The remaining tubes confirmed this statement).

Tubes 16, 17, 18, 19 gave results identical with those given by tubes 11, 12, 13, 14, again proving that the auto-hemolysin of hemoglobinuric serum is capable of dissolving an individual's corpuscles for which the serum contains an iso-hemolysin.

Having absorbed the iso-hemolysin from hemoglobinuric serum and left the autohemolysin, it seemed desirable, in order to complete this part of the study, to absorb the auto-hemolysin from hemoglobinuric serum and leave the iso-hemolysin. This was readily accomplished in the following manner:

Blood was obtained from—

No. 1. A normal individual belonging to Group I.

PROTOCOL III.

To absorb the auto-amboceptor from hemoglobinuric serum leaving the iso-amboceptor.

1. Ser. 5, ¼ cc. + Corps. 1, ¼ cc. 37°C. 2 hrs. = + hem. 0°C. ½ hr. 37°C. 2 hrs. = + hem.
2. Ser. 5, ¼ cc. + Corps. 4, ¼ cc. 37°C. 2 hrs. = 0 hem. 0°C. ½ hr. 37°C. 2 hrs. = + hem.
3. Ser. 5, ¼ cc. + Corps. 5, ¼ cc. 37°C. 2 hrs. = 0 hem. 0°C. ½ hr. 37°C. 2 hrs. = + hem.
4. Ser. 4, ¼ cc. + Corps. 5, ¼ cc. 37°C. 2 hrs. = 0 hem. 0°C. ½ hr. 37°C. 2 hrs. = 0 hem.
5. Ser. 5 ina., ¼ cc. + Corps. 1, ¼ cc. 37°C. 2 hrs. = 0 hem. 0°C. ½ hr. 37°C. 2 hrs. = 0 hem.
6. Ser. 5 ina., ¼ cc. + Corps. 5, ¼ cc. 0°C. ½ hr. 37°C. 2 hrs. = 0 hem.
7. Ser. 5 ina., ¼ cc. + Corps. 1, ¼ cc. + Comp. (ser. 4) 1/10 cc. 37°C. 2 hrs. = + hem.
8. Ser. 5 ina., ¼ cc. + Corps. 5, ¼ cc. + Comp. (ser. 4) 1/10 cc. 0°C. ½ hr. 37°C. 2 hrs. = + hem.
9. Ser. 5, 1 cc. + Corps. 5, 1/20 cc. 0°C. ½ hr. Centrifugalize at 0°C. same serum and wash corpuscles three times at 0°C.
10. One-half the washed corps. from tube 9 + NaCl 0.45 cc. 37°C. 2 hrs. = 0 hem.
11. One-half the washed corps. from tube 9 + NaCl 0.45 cc. + Comp. (ser. 4) 1/10 cc. 37°C. 2 hrs. = + hem.
12. Ser. tube 9, ¼ cc. + Corps. 5, ¼ cc. 0°C. ½ hr. 37°C. 2 hrs. = 0 hem.
13. Ser. tube 9, ¼ cc. + Corps. 5, ¼ cc. + Comp. (ser. 4) 1/10 cc. 0°C. ½ hr. 37°C. 2 hrs. = 0 hem.
14. Ser. tube 9, ¼ cc. + Corps. 5, ¼ cc. + Ser. 5 ina., ¼ cc. 0°C. ½ hr. 37°C. 2 hrs. = + hem.
15. Ser. tube 9, ¼ cc. + Corps. 1, ¼ cc. 37°C. 2 hrs. = + hem.

Wherever inactive serum was used in the above experiments it is indicated by the abbreviation "ina." Elsewhere active serum was used.

The corpuscles were used in 5% suspension except in tube 9, where all the supernatant salt solution after centrifugalization was removed from the corpuscles before they were used.

No. 4. A normal individual belonging to Group IV.

No. 5. Case I, G. C., hemoglobinuric patient, Group IV.

Tubes 1 to 8 inclusive are controls and show as follows:

Tube 1 shows that serum 5 contains an iso-hemolysin for corpuscles 1 (reading at the end of first 2 hours at 37° C.).

Tubes 2 and 3 show that serum 5 contains an auto-hemolysin for corpuscles 4 and 5 (reading after ½ hour at 0° C. and 2 hours at 37° C.).

Tube 4 shows that serum 4 contains neither iso- nor auto-hemolysin for corpuscles 5, therefore it can be used for complement, provided it is able to reactivate serum 5 inactive.

Tube 5 shows that both the iso- and auto-hemolysin contained in serum 5 for corpuscles 1 were inactivated by heating to 56° C. for 20 minutes.

Tube 6 shows that the auto-hemolysin contained in serum 5 for corpuscles 4 was inactivated by heating to 56° C. for 20 minutes.

Tubes 7 and 8 show that serum 4 (complement) is capable of reactivating both the iso- and auto-amboceptor contained in serum 5 inactive.

Tube 9 was to absorb the auto-amboceptor from serum 5 by means of corpuscles 5 at 0° C. Hemolysis was prevented by keeping the mixture at a low temperature until after the serum had been removed from the corpuscles and the latter thoroughly washed with salt solution.

Tube 10 shows that the corpuscles did not anchor, at least not permanently, both auto-amboceptor and complement at 0° C.

Tube 11 shows that the corpuscles did anchor the auto-amboceptor at 0° C., that dissociation did not occur as a result of thorough washing of the corpuscles and that complement united at 37° C. with consequent solution of the corpuscles. This and tubes 12, 13, 14 are a repetition and confirmation of experiments done many times under subhead 3; namely: Determination of the relation of temperature to the union of (a) amboceptor and corpuscle (b) complement and amboceptor-corpuscle.

Tube 12 shows that serum 5 after absorbtion with corpuscles 5 in tube 9 at 0° C. was unable to dissolve corpuscles 5.

Tube 13 shows that the failure of hemolysin in tube 12 was not due to lack of complement.

Tube 14 shows that the failure of hemolysis in tube 12 was due to lack of auto-amboceptor only.

Tube 15 shows that the auto-amboceptor was absorbed from serum 5 in tube 9, leaving iso-amboceptor and complement, Q. E. D.

Hoover and Stone were able to absorb the auto-amboceptor from hemoglobinuric serum in the absence of complement, as indicated by the following quotation, to which reference has previously been made: "Thus we have shown that if in two stages, 10 drops of a 10% suspension of red cells be added to (1 cc.) the serum and exposed to cold for one hour each time, the amboceptor can be exhausted from the inactivated hemoglobinuric serum." It seems probable that this result is to be explained on the basis of a mechanical removal due to the relatively large amount of corpuscles used. Even so they found it necessary to absorb in two stages.

In my efforts to separate the auto- and iso-amboceptor in hemoglobinuric serum I found it necessary to use the minimum quantity of corpuscles which would completely remove the one in order that the other be left. In case an excess of corpuscles was employed both auto- and iso-amboceptor would be removed.

5. *Resistance of the red blood corpuscles.*

Certain investigators have observed that the red blood cells of hemoglobinuric patients studied by them were less resistant to hypotonic salt solution than normal, while other investigators have found no difference between normal corpuscles and the corpuscles of their patients in relation to hypotonic salt solution. Dr. Helen Watson kindly tested this point for me in my three cases. The corpuscles of Cases I and II were tested on two occasions, an interval of about two months elapsing between tests. The corpuscles of Case III were only

tested once. The corpuscles of all three patients showed definitely increased resistance to hypotonic salt solution. The only exception to this rule was Case II, whose corpuscles, at the time of the second test, showed the same resistance as those of several normal individuals, tested at the same time, but on the occasion of the first test they showed a markedly greater resistance than normal corpuscles.

It has been shown by numerous investigators that the destruction of erythrocytes during the paroxysms of this disease is not due to any inherent weakness of the cells but to a peculiar action of the serum of these patients and that this serum is capable of dissolving not only the corpuscles of hemoglobinuric patients, but also those of normal individuals. With the exception of the variation of resistance of hemoglobinuric corpuscles I know of no reason why they may not be regarded as normal corpuscles. That they show a variable and sometimes greater than normal resistance may possibly be explained in the following way.

It seems reasonably certain that the individual corpuscles of any person differ in their resistance. This idea is based on the fact that if one sets up a series of tubes containing falling strengths of salt solution (3 cc. in each tube) and introduces one drop (approximately 1/30 cc.) of corpuscles into each tube, it will be observed that hemolysis begins in a dilution of about 0.475% NaCl but is not complete until a dilution of about 0.30% is reached. That is, in the tube containing 0.475% NaCl only a few of the introduced corpuscles are dissolved. That these few are dissolved and the others are not seems to be best explained on the assumption that those corpuscles which were dissolved were less resistant to the action of hypotonic salt solution than the remainder of the corpuscles. That the small amount of serum (or iso-tonic salt solution) introduced with the drop of corpuscles plays but little part in altering the tonicity of the mixture is evident from the proportion used, 3 cc. 0.475% NaCl + 1/30 cc. 0.85% NaCl gives 3 1/30 cc. of a 0.479% solution. The slight increase of tonicity of the 0.475% solution due to the addition of the salt carried over with the one drop of corpuscles and to the solution of a part of the corpuscles, might protect the remainder of the corpuscles, but this explanation cannot serve in the case of a 0.40% salt solution where only a portion of the added corpuscles undergoes solution. In this case 1/30 cc. 0.85% NaCl added to 3 cc. of 0.40% NaCl brings the concentration of the resulting mixture only up to 0.405%, which is still relatively far below that in which hemolysis first appeared (0.475%). In the above examples the entire drop of corpuscles has been considered as the equivalent of 0.85% salt solution, whereas it probably has something less than this value in raising the tonicity of the mixture.

Assuming that individual corpuscles of a person vary in their resistance to hypotonic salt solution, it seems not unlikely that they differ in their resistance to other destructive agents, and that during an attack of paroxysmal hemoglobinuria those corpuscles possessing the least resistance would be the ones destroyed, leaving the more resistant ones. If this theory is correct, a resistance test taken shortly after an attack of hemoglobinuria would show increased resistance, while a test taken at a considerable interval after an attack would show normal relations. The correctness of this theory remains to be proven.

6. *The Wassermann Reaction.*

In the reported cases of paroxysmal hemoglobinuria, evidence of syphilis, usually congenital, has been observed so often as to give weight to the suggestion frequently made that the latter perhaps stands in an etiological relationship. All efforts to find an infectious agent in the blood of these patients have, so far as I am aware, failed. The idea advanced that the disease is due to an auto-immunization resulting from a resorption by the patient of his own blood has never been proven and there is but scant evidence in its support. Of the various suggestions made that of congenital syphilis seems to have most evidence in its favor.

Dr. C. R. Austrian kindly did the Wassermann reaction of the three cases here reported. All gave positive reactions. Tests were done on both the father and mother of Case I, and the mother was found also to give a positive reaction. The father of Case II gave a negative reaction and although the mother was not tested, the history of four miscarriages in the mother is suggestive.

Case III gave no history of syphilis, but had, as stated, a positive Wassermann reaction.

*7. The effect of the administration of Ehrlich's "606," (a) on the Wassermann reaction; (b) on the clinical course of the disease.*

On November 15, 1911, Case I was given 0.3 gram "606" intravenously. She remained in the hospital until December 8, 1910. During most of this time she was kept in bed and not exposed to a sufficient degree of cold to induce an attack of hemoglobinuria. Nevertheless, on December 3, without premonitory symptoms, she voided a dark brown urine which gave a positive guiac test for blood, as is stated in the case history. The Wassermann reaction was still positive.

After the child's discharge from the hospital, she had no further attack until about March, 1911. The freedom from attacks during the interval, the patient, since the onset of the disease, never having gone so long during cold weather without attacks, led the mother to believe that a cure had been effected. Accordingly, of her own initiative and without my knowledge, she frequently sent the child out to play during cold, damp weather, without hat or coat, in order to see if an attack would be induced. Notwithstanding this marked improvement in the clinical condition, the patient's serum is still able, in vitro, to hemolyse her own and other corpuscles, when subjected to the cold-warm test, although not to the same degree, I think, as before the administration of "606," and she still has a positive Wassermann reaction.

It seems advisable to repeat the administration of "606" in this case, and I hope to have the opportunity of trying it on the other two cases before the onset of cold weather.

## Summary.

The serum of patients suffering from paroxysmal hemoglobinuria contains a complex hemolysin, of amboceptor-complement nature, which is capable of bringing about the

solution of the patient's own corpuscles, corpuscles of other paroxysmal hemoglobinuric patients, and of all other individuals, as far as tested.

Patients suffering from paroxysmal hemoglobinuria are not confined to one group, as determined by the iso-agglutination reaction, and their serum may contain normal iso-hemolysin in addition to the hemolysin characteristic of their disease.

The auto-amboceptor may be absorbed from patient's serum, leaving the iso-amboceptor and conversely the iso-amboceptor may be absorbed, leaving the auto-amboceptor, thus enabling each to be tested separately on any given set of corpuscles.

Only the amboceptor component of the hemolysin of paroxysmal hemoglobinuria is peculiar to the disease. The complement differs in no way, so far as tested, from that present in normal serum.

The amboceptor peculiar to paroxysmal hemoglobinuria differs from other known hemolytic amboceptors in that it will unite with the red blood corpuscles only at a low temperature in the presence of complement, and furthermore, in that it is capable of bringing about the solution of the patient's own cells (auto-hemolytic action) and those of other members of the group to which the patient belongs, as well as the cells of members of other groups.

Hemolysis due to the auto-hemolysin of paroxysmal hemoglobinuria unlike normal iso-hemolysis, may occur entirely independently of agglutination.

The red cells of the three cases here reported showed a variable and usually increased resistance to hypotonic salt solution. Never a resistance less than that of normal corpuscles.

All three cases gave a positive Wasserman reaction.

Case I received 0.3 gram Ehrlich's "606" in November, 1910. Prior to that time the patient, never, since the onset of the disease, went more than two or three weeks, except during summer, without an attack of hemoglobinuria, and often had attacks at shorter intervals. Since receiving "606," the Wassermann reaction has remained positive, but the patient has, up to the present time, May, 1911, suffered but two attacks, although repeatedly exposed to cold.

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# THE CALCIFICATION OF THE COSTAL CARTILAGES, THE CARDIO-THORACIC INDEX AND OTHER SIGNS OF PULMONARY TUBERCULOSIS.

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The radiographic diagnosis of pulmonary tuberculosis is based upon more or less characteristic alterations in the shadows of the hilum and lung fields, and by some radiographers also upon alterations in the shadow of the heart, diaphragm and thoracic walls. The alterations in the shadows of the hilum and lung fields are dependent upon morbid changes present in the lungs and hilum, as a result of the local activity of the tubercle bacilli. The alterations in the shadows of the heart, diaphragm and thoracic walls are due to changes in these structures, and are generally considered as either secondary changes, or as predisposing factors to the pulmonary disease. In a previous article[1] we have considered at length the appearance and diagnostic value of the shadows of the hilum and lung fields. At this time we wish to consider the nature and diagnostic value of the alterations in the shadows of the heart, diaphragm and thoracic walls.

On reviewing the literature one finds that there are many alterations in the shadows of these structures, to which more or less importance has been attached. The most frequently mentioned are the "small pendulous heart," and the calcification of the rib cartilages. Other alterations noted are narrow interspaces, contraction of one side of the thoracic wall, decrease in the angle made by the neck muscles and the clavicle, various abnormalities in the shape of the upper aperture of the chest cavity, alterations in the outline and height of the diaphragm, etc.

For the studies here reported, the radiographs taken in

[1] See first paper in this number.

connection with the stereo-rœntgenographic work done in the Research Laboratory of the Phipps Dispensary of the Johns Hopkins Hospital, were used. In taking these plates the relative position of X-ray tube, patient, and photographic plate was the same in every case. As previously reported, each patient radiographed was subjected to careful clinical examination and the conclusions, independently arrived at by these different methods of examination, compared. In this series only those cases are considered in which there was complete agreement in the findings by both clinical and radiographic examination.

Turning now to a consideration of the heart in pulmonary tuberculosis, we wished to determine, if possible, the occurrence of any characteristic alteration in the heart shadow which might be of diagnostic value. As previously stated, the so-called "small pendulous heart" is most frequently mentioned by radiographers as a sign of value. By some it is considered as merely indicating a marked predisposition to pulmonary tuberculosis; by others, however, it is considered a change secondary to tuberculous infection of the lungs. In studying this question a series of ninety-eight cases was examined, in which the clinicians had given special attention to the condition of the heart. As a basis of comparison of individual cases or of groups of cases, it is evident that the actual area of the cardiac shadow would be valueless, as this must vary with the size, age, sex, etc., of the patient. It was, therefore, necessary to consider the size of the heart shadow in comparison with the size of some other shadow, the original of which varies in the same way as does the heart with the size, age, sex, etc., of the patient. To this end the greatest transverse diameter of the heart shadow was compared with the greatest transverse diameter of the chest shadow and the resulting ratio, which therefore expresses the size of the heart in relation to the size of the chest, was termed the Cardio--Thoracic Index.

TABLE I

SHOWING THE INFLUENCE OF SEX UPON THE CARDIO-THORACIC INDEX.

| | | Cardio-Thoracic Index | | |
|---|---|---|---|---|
| Sex | No. of Cases | Average | Max. | Min. |
| Male | 47 | 0.446 | 0.52 | 0.35 |
| Female | 33 | 0.430 | 0.50 | 0.37 |

Table I shows that in the cases examined the average cardio-thoracic index, independent of age or physical condition, was slightly greater in the males than in the females.

TABLE II

SHOWING THE INFLUENCE OF AGE UPON THE CARDIO-THORACIC INDEX.

| | | Cardio-Thoracic Index | | |
|---|---|---|---|---|
| Age | No. of Cases | Average | Max. | Min. |
| 0-5 years | 3 | 0.456 | 0.47 | 0.39 |
| 5-15 years | 11 | 0.446 | 0.50 | 0.41 |
| 15-30 years | 45 | 0.450 | 0.50 | 0.37 |
| 30-40 years | 15 | 0.443 | 0.52 | 0.39 |
| 40-50 years | 4 | 0.42 | 0.52 | 0.40 |
| 50-60 years | 2 | 0.42 | 0.45 | 0.40 |

Table II shows the cardio-thoracic index, independent of sex or physical condition, to be practically constant in the different age periods, with a slight tendency to decrease in later life.

TABLE III

SHOWING THE INFLUENCE OF PULMONARY TUBERCULOSIS UPON THE CARDIO-THORACIC INDEX.

| | | Cardio-Thoracic Index | | |
|---|---|---|---|---|
| Stage of Disease | No. of Cases | Average | Max. | Min. |
| Neg. | 14 | 0.452 | 0.50 | 0.35 |
| Doubt. | 16 | 0.440 | 0.48 | 0.39 |
| I Stage | 6 | 0.435 | 0.46 | 0.41 |
| II Stage A | 14 | 0.437 | 0.50 | 0.37 |
| II Stage B | 13 | 0.449 | 0.48 | 0.40 |
| III Stage | 17 | 0.438 | 0.52 | 0.39 |

Table III is of special importance. As will be seen, the cases are divided into Non-Tuberculous, Doubtful and Tuberculous. The Tuberculous are sub-divided into four groups depending on the stage of the disease,—first stage, early second stage, late second stage and third stage. Here the cardio-thoracic index is practically constant in the different groups, with, however, a slight tendency for the average to be smaller in the tuberculous than in the non-tuberculous and doubtful cases. However, one fact must be borne in mind, and this is, that the cardio-thoracic index varies between fairly wide limits in the individual cases, both normal and tuberculous, as is shown by the maximum and minimum indices given in the various tables.

TABLE IV

SHOWING THE INFLUENCE OF CARDIO-VASCULAR DISEASE UPON THE CARDIO-THORACIC INDEX.

| | | Cardio-Thoracic Index | | |
|---|---|---|---|---|
| Disease | No. of Cases | Average | Max. | Min. |
| Myocarditis | 5 | 0.564 | 0.59 | 0.54 |
| Mitral Insufficiency | 7 | 0.502 | 0.57 | 0.43 |
| Aneurism | 6 | 0.543 | 0.58 | 0.52 |

Table IV shows a high cardio-thoracic index in 18 cases with definite cardiac lesions. These cases naturally are not included in the previous tables.

From our pathological experience we know that a small heart is a common finding in individuals dead of chronic wasting disease, such as tuberculosis, carcinoma, etc. Here the small heart is merely an expression of the general wasting and is not characteristic of any special disease process. Thus in one of our cases, dead of carcinoma of the oesophagus, the radiograph taken just before the autopsy showed a cardio-thoracic index of 0.37. However, in our third stage cases (Table III) which were all ambulatory, the average cardio-thoracic index was 0.438 with the maximum 0.52 and a minimum of 0.39. Evidently, then, the small heart is far from constant even in third stage cases. Now as the value of the radiographic examination must depend upon one's ability to discover disease processes at a time when the clinician is still doubtful, or anxious for confirmation of his findings, it is use-

less to attempt to base a diagnosis upon changes which are neither constant nor characteristic, even in the late stages of the disease.

From the preceding we may safely conclude that the average cardio-thoracic index is practically constant in the different age periods, that it is slightly smaller in females than in males, and that in the tuberculous, although on the average it shows a very slight tendency to be somewhat smaller than in the non-tuberculous, this average tendency is so slight and varies so widely in individual cases, that it cannot be considered a sign of any value in the radiographic diagnosis of pulmonary tuberculosis.

The pendulous character of the heart shadow, sometimes described as significant of pulmonary infection, is dependent for its appearance upon the small size of the heart and must therefore share with the cardio-thoracic index the same lack of value as a diagnostic sign.

Regarding the occurrence and diagnostic value of calcification of the rib cartilages, two theories have been advanced. The one that calcification, by interfering with the free movement of the thoracic walls, renders proper aeration of the apices impossible and therefore acts as a strong predisposing factor to pulmonary tuberculosis, the other that the calcification is secondary to the pulmonary disease and depends upon altered metabolic processes. Pathological experience seems to favor the latter view. In tables V-VII, the results of the investigation of 153 cases are tabulated. Cases showing complete calcification of the first cartilage, with or without calcification of the other cartilages, are designated positive. Cases showing partial calcification of the first cartilage, with or without partial calcification of the other cartilages, are designated slight.

TABLE V

SHOWING THE INFLUENCE OF SEX UPON THE CALCIFICATION OF THE RIB CARTILAGES.

| | | Calcification | | |
|---|---|---|---|---|
| Sex | No. of Cases | % + | % Slight | % — |
| Male | 85 | 30 | 22 | 48 |
| Female | 68 | 17 | 17 | 66 |

Table V shows that calcification was more common in the male cases, independent of age and physical condition.

TABLE VI

SHOWING THE INFLUENCE OF AGE UPON CALCIFICATION OF THE RIB CARTILAGES.

| | | Calcification | | |
|---|---|---|---|---|
| Age | No. of Cases | % + | % Slight | % — |
| 0-5 years | 6 | 0 | 0 | 100 |
| 5-15 years | 20 | 0 | 0 | 100 |
| 15-30 years | 78 | 19 | 17 | 64 |
| 30-40 years | 29 | 33 | 33 | 34 |
| 40-50 years | 9 | 40 | 47 | 13 |
| 50-60 years | 9 | 68 | 32 | 0 |
| 60-70 years | 2 | 50 | 50 | 0 |

Table VI shows that calcification ran parallel with the age.

TABLE VII

SHOWING THE INFLUENCE OF PULMONARY TUBERCULOSIS UPON CALCIFICATION OF THE RIB CARTILAGES.

| | | Calcification | | |
|---|---|---|---|---|
| Stage of Disease | No. of Cases | % + | % Slight | % — |
| Neg. | 37 | 36 | 28 | 36 |
| Doubt. | 35 | 10 | 27 | 63 |
| I Stage | 9 | 0 | 10 | 90 |
| II Stage A | 37 | 19 | 22 | 59 |
| II Stage B | 16 | 22 | 7 | 71 |
| III Stage | 19 | 46 | 5 | 54 |

Table VII shows that calcification was present in a large percentage of the non-tuberculous cases. It also shows that calcification was absent in the first stage of the disease and increased in frequency with advance in the disease; however, only in the third stage cases did the percentage of positive cases exceed that found in the non-tuberculous cases. It might be mentioned that the patients comprising the third stage group were slightly older than those comprising the non-tuberculous group.

From these tables we may conclude that calcification of the rib cartilages, especially the first, is more common in males than in females, that it increases in frequency with advancing years and finally, that its incidence in pulmonary tuberculosis is only accidental, or a late secondary change, and that its occurrence is of no diagnostic significance whatever in the individual case.

The size of the aortic shadow was studied in relation to the size of the heart but nothing of importance in regard to the diagnosis of pulmonary tuberculosis was determined.

TABLE VIII

SHOWING THE ABSENCE OF ANY RELATION BETWEEN THE WIDTH OF THE 2ND INTERSPACE AND PULMONARY TUBERCULOSIS.

| | | Width of 2nd Interspace | | |
|---|---|---|---|---|
| Stage of Disease | No. of Cases | Average | Max. | Min. |
| Neg. | 36 | 3.0 cm. | 4.4 cm. | 1.5 cm. |
| Doubt. | 35 | 2.7 cm. | 4. cm. | 1.8 cm. |
| I Stage | 9 | 2.9 cm. | 3.8 cm. | 2.2 cm. |
| II Stage A. | 37 | 2.9 cm. | 4.5 cm. | 2. cm. |
| II Stage B. | 16 | 3.3 cm. | 4. cm. | 2.5 cm. |
| III Stage. | 20 | 2.8 cm. | 4. cm. | 1.8 cm. |

Another sign to which attention is sometimes called is the presence of narrow interspaces in individuals suffering from pulmonary tuberculosis. The width of the 2nd interspace in the left midclavicular line was measured and the results recorded in table VIII. From this it is seen that no apparent relation exists between the width of this interspace and the presence or absence of pulmonary infection. Whether we may take the width of the 2nd interspace as a basis for comparison is, however, open to argument.

TABLE IX

SHOWING THE ABSENCE OF ANY RELATION BETWEEN THE ANGLE OF THE 6TH RIB AND PULMONARY TUBERCULOSIS.

| Stages of Disease | No. of Cases | Angle of 6th Rib | | |
|---|---|---|---|---|
| | | Average | Max. | Min. |
| Neg. | 36 | 100° | 104 | 85 |
| Doubt. | 35 | 97° | 103 | 99 |
| I Stage | 9 | 101° | 105 | 97 |
| II Stage A | 37 | 99° | 104 | 97 |
| II Stage B | 16 | 100° | 103 | 80 |
| III Stage | 22 | 97° | 109 | 85 |

No very absolute measurements could be made of the angle of the ribs, but in table IX we have recorded the average angle made by the spine and the sixth rib on the left side. Apparently no relation exists between this angle and pulmonary tuberculosis.

The position in which our cases were radiographed made it impossible to study the shape of the upper aperture and alterations in the angles of the neck muscles.

No definite conclusions were reached from our study of the height of the diaphragm, since the average height was about the same in the tuberculous and non-tuberculous cases and the variation in individual cases was very great. There are, however, several interesting features about the diaphragm shadow, especially the occurrence of irregularities, which are worthy of more extended consideration, especially in connection with post mortem examinations.

In conclusion, we believe we are justified in saying that these signs—small pendulous heart, calcified cartilages, narrow interspaces, and excessive sloping of the ribs—are not only valueless, but are absolutely misleading, if considered as positive signs in the radiographic diagnosis of pulmonary tuberculosis.

# EXPERIMENTAL STUDIES ON TUBERCULO-PROTEIN HYPERSENSITIVENESS AND THEIR POSSIBLE APPLICATIONS.[1]

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It gives me great pleasure to appear before the Laennec Society as an envoy of the laboratory whose founder, Dr. Edward L. Trudeau, has so often acknowledged his indebtedness to the men of the Johns Hopkins Medical School, who by their generous advice and assistance contributed in no small measure to the success of his work at Saranac Lake.

Ever since Koch's discovery of tuberculin it has been universally taught and believed that the tuberculous animal differs fundamentally from the non-tuberculous animal in its response to injections of derivatives of the tubercle bacillus. The tuberculous animal becomes acutely ill several hours after a subcutaneous injection of tuberculin: it suffers from weakness and prostration and a rise of temperature, and at the same time there are evidences, such as increased cough and expectoration in pulmonary disease, or swelling, pain and redness in joint or glandular involvement, that there is an exacerbation of the disease at the focus. Besides this, at the site of injection there is frequently an area of redness, swelling and pain. The tuberculous animal is therefore found to react to tuberculin in three different ways, to which the terms general, focal, and local or puncture reactions have been applied. It was also discovered by Babes and Proca that if tubercles were induced in an animal by the intravenous injection of dead tubercle bacilli, such animals would likewise react to tuberculin with general, focal, and local signs and symptoms. The general reaction is in both instances considered to be some kind of an intoxication, while the focal and local reactions are forms of inflammation.

Such animals were therefore said to be specifically sensitive to tuberculin; and, as our knowledge of the products of the bacillus extended, it was found that practically every preparation that contained bacillary protein could bring about reactions in animals that harbored tubercles set up by living or dead bacilli. Bacillary emulsion, T. O., T. R., water extract, bacillary filtrate, precipitated tuberculin and a host of other preparations were all like old tuberculin in that extremely small amounts of them rendered the animals ill.

At the same time it was noted that healthy, non-tuberculous animals could sustain relatively enormous doses of tuberculin and tuberculo-derivatives without harm. Although some workers, particularly Landmann, by methods of concentration succeeded in obtaining from tubercle bacilli products that would poison the healthy animal in moderate amounts, still the non-tuberculous always resisted much larger doses of any given preparation than the tuberculous. The healthy animal was accordingly considered to be insensitive to tuberculin and its related products. More than this, the claim was generally made that the animal without specific tubercles could not in any way be made sensitive to tuberculo-protein. Only within the last year no less an authority than Hamburger has come to this conclusion, and within the past two or three months, Kraus, Loewenstein and Volk have supported his contention on experimental evidence, while Wassermann, Pappenheim and von Pirquet have recently voiced the same assumption.

Since every theory that has tried to explain the so-called tuberculin reaction is based on evidence that has denied the possibility of sensitizing non-tuberculous animals with derivatives of the bacillus, Baldwin's announcement, made a year ago, that he had succeeded in doing just this thing was of the greatest importance. It is this work and further ap-

[1] Address delivered before the Laennec Society, Johns Hopkins Hospital, April 24, 1911.

plications of it and the bearing they may have on some of the problems of tuberculosis that I wish to go over with you to-night.

Three years before Baldwin, in 1907, Rosenau and Anderson had intoxicated previously sensitized guinea-pigs with an extract of human tubercle bacilli, so that after a subcutaneous or intraperitoneal injection a few of their animals showed mild symptoms of illness that consisted of restlessness, scratching the nose, irregular respiration, tremor and weakness. They did not follow up this work with further experimentation.

Baldwin sensitized guinea-pigs by the ordinary methods of intraperitoneal or subcutaneous injections of a derivative of the tubercle bacillus. As a rule his preparation was a water-extract-filtrate of the micro-organisms. He obtained this by digesting at 50° C. in distilled water the powder derived from thoroughly washed, dried, pulverized cultures, that had been grown in broth media. After extracting the powder for a day or two, he filtered the supernatant extract-emulsion through a Berkefeld bougie. The filtrate was a material that was freed from bacilli and which very likely contained no other organic constituents except bacillary proteins. Since the organisms were very carefully washed when they were separated from the culture media it is hardly possible that any protein constituents of the broth were carried over with the bacilli except in very negligible quantity. By using this material the contingency of a broth protein sensitization was avoided, and the results of the subsequent experiments could be interpreted as being due to the effect of bacillary protein.

If now a normal animal was treated with a single dose of a small amount of such a water-extract it was found that two or three weeks later it would respond to a second or toxic injection of bacillary protein with all the symptoms of acute anaphylaxis, dying as a rule in from three to six minutes. The material that was used for this second or toxic injection was usually the unfiltered, supernatant extract-emulsion which I have already mentioned. This is the product which Koch described at T. O. It is an extract of the bacilli, and, since it contains the ground-up bodies of the organisms, it is an emulsion as well: we have for this reason usually designated it as extract-emulsion in our work.

Although Baldwin and I found that a small amount of tuberculo-protein would sensitize an animal if it was introduced by almost any parenteral route, it soon appeared that not every way was available for the second injection if toxic symptoms were to be elicited. Baldwin practised toxic injections by way of the peritoneum and subcutaneous tissue without result, and on account of operative shock he got obscure or questionable results with intracerebral and intracardial injections. By using the postorbital method of injection, which had been already described by Gay and Southard, he succeeded in producing acute anaphylaxis almost invariably in sensitized animals. The symptoms were sharp and definite. For a minute or so following the injection the animal was apparently well and normal. It then began to scratch its nose, to cough spasmodically, and to go through "bucking" movements. Dizziness, convulsions and coma rapidly succeeded each other and in a very few minutes the animal was dead. Respiratory distress dominated the picture throughout the attack. At first deep and labored and perceptibly slowed, the thoracic excursions became greater and greater and more and more delayed, until approaching death was signalized by a few tremendous inspirations which toward the end were as much as thirty or forty-five seconds apart. There was no mistaking the character of the symptoms. They were an exact replica of what had been so often described as the anaphylactic shock caused by other proteins. Nor were the pathological findings any less typical. All the animals, that died in this way, had the large, white, blown-out lungs that Auer and Lewis had proved to be characteristic, the pathological anatomy of which has so recently been worked out by Schultz and Jordan. The epicardial hemorrhages to which attention was called by Gay and Southard were also a constant feature.

The amounts that Baldwin usually used were one or two cubic centimeters of water extract intraperitoneally to sensitize a full-grown guinea-pig, and one-half to one cubic centimeter of extract-emulsion postorbitally to intoxicate it fatally. I found later that I could satisfactorily sensitize an animal with 0.01 cc. water extract given intradermically, bring on fatal anaphylaxis with 0.05 cc. extract-emulsion postorbitally, and cause symptoms with 0.03 cc. extract-emulsion postorbitally. The last named quantities are represented by 0.05 mg., 0.9 mg. and 1.6 mg. dry weight respectively.

Such experiments showed us at once that we had succeeded in putting non-tuberculous animals in such a condition that in one respect they resembled tuberculous guinea-pigs: that is, that a fraction of a milligram of tuberculo-protein would make them violently ill. Let us see how the similarity worked out after further experimentation. Let us remember too, that up to this point our information comprised the following facts: the normal, non-tuberculous animal can tolerate the injection of large amounts of our tuberculo-preparations—1.0 cc. extract-emulsion postorbitally, at least 5.0 cc. intravenously, and at least 10 or 15 cc. intraperitoneally without effect; less than a milligram postorbitally will bring about an acute and fatal intoxication in a sensitized non-tuberculous animal; and a fraction of a milligram, introduced subcutaneously or intraperitoneally, will kill a tuberculous guinea-pig with a focal and general reaction that comes on several hours after the injection of the antigen.

I have said that Baldwin failed to poison sensitized guinea-pigs by intraperitoneal injections. As a general thing it is impossible to do so, but in a few very highly sensitive animals I have succeeded in inducing symptoms of illness. I used moderate-sized toxic doses. A citation of a couple of the experiments will show what occurred.

**ANIMAL 1.—Sensitized 82 days previously. Injected intraperitoneally with 1.5 cc. E. E. 161.**

***Result.*—The animal seems normal and runs around for about 10 minutes after the injection. Then symptoms of illness begin to develop rapidly. It drags its hind legs, becomes very weak and lies down on its side. These symptoms continue for 10 minutes,**

when they begin to subside. Within a half hour after the injection the animal has almost regained its normal condition.

ANIMAL 2.—Sensitized 126 days previously. Injected intraperitoneally with 2.5 cc. E. E. 161.

*Result.*—No symptoms for 10 minutes. Then the animal becomes weak and staggers around on the floor. The respiration is slow and labored with marked heaving of the chest. The animal slowly recovers from the symptoms.

Here in certain selected animals I get symptoms of illness from an intraperitoneal injection. The fundamental character of the reaction must be the same as that which occurs when I inject postorbitally. But the reaction expresses itself differently. In the latter case, there is a fulminant process that ends in death in a very few minutes; in the former, there is a prolonged chain of symptoms that come on gradually, are much less severe and terminate in recovery. If, now, I make an intravenous, toxic injection on a sensitized animal, I find that it will have the same result as a postorbital one. If, however, I inject subcutaneously, I elicit no symptoms. I am taught, therefore, that given a sensitive animal, the nature of its response to a toxic injection of tuberculo-protein will depend altogether on my method of introducing the antigen. If the preparation enters the body in such a way that it will get into the circulation rapidly, acute and fatal anaphylaxis will result. If it is absorbed more slowly, there will either be no symptoms or the symptoms will tend to approximate the comparatively gradual illness that occurs when we inject the tuberculous animal subcutaneously. It further seems as though we have to do with an antibody that is present in the circulation which reacts with the antigen to set free a powerful toxin—the so-called anaphylotoxin of Friedberger. In further support of this idea that the antibody that is concerned in the production of the acute anaphylaxis is free in the circulation, is the circumstance that in some forms of protein anaphylaxis, the serum of a sensitized animal will render a second animal sensitive to the particular protein if transferred to the latter.

After demonstrating that the non-tuberculous animal that had been treated with tuberculo-protein was unlike the normal animal but similar to the tuberculous animal in that it would react to very small amounts of this antigen, Baldwin showed further that a large proportion of tuberculous animals that had never before received tuberculo-protein would react to it exactly like the sensitized animals that were not tuberculous. If he injected such tuberculous animals subcutaneously or intraperitoneally with extract-emulsion, they would suffer the well-known general tuberculin reaction, which manifested itself several hours later and ended in death if the dose was sufficiently large. If, however, he introduced his extract-emulsion postorbitally, many of these tuberculous animals would die in a few minutes of acute anaphylaxis and would show the typical lesions of the heart and lungs. Not all tuberculous animals, picked at random, will react in this way and the reason why they differ from one another is not yet clear, but probably depends upon the nature and extent of the tuberculous foci, the conditions of absorption from them, and the animals' inherent ability to create antibodies. But after injecting say fifteen tuberculous guinea-pigs of one lot, I have had sixty per cent show acute anaphylaxis.

This event brought out plainly that all that was necessary to prove one point of similarity between tuberculous guinea-pigs and sensitized, non-tuberculous ones was to treat each kind with a toxic dose of antigen postorbitally—that is, in a rapidly absorbable way. The first logical deduction was that the tuberculous animal had a circulating anaphylactic antibody; another, that it was identical with that of the sensitized, non-tuberculous animal; a third, that it was produced in response to the same antigen as in the latter; and a fourth, that the antigen arose in the tuberculous focus and was probably the tubercle bacillus.

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After these relations had been worked out the first problem that naturally suggested itself was to find out what would happen to a non-tuberculous animal if it were treated with tuberculo-protein like tuberculous animals that are undergoing tuberculin therapy. The specific treatment of tuberculosis after a long period of disuse and even disrepute has of late years been gradually coming into its own again. Clinician after clinician is now taking up the method, and the majority of capable observers are reporting strikingly good results in many cases. However, the action of the bacillary preparations so far as their therapeutic uses are concerned still remains as mysterious as ever. Their application thus far has been purely empirical and limited only by the clinical judgment of the practitioner. The general opinion of the latter has been, I believe, that the introduction of carefully graded doses rouses the tuberculous organism to the overproduction of defensive substances—antibodies—that neutralize the poisons elaborated in the focus or attack the bacilli themselves or perform both of these functions.

The idea that the bacillary products that are ordinarily used contain primary toxins, does not seem tenable. If we had to do with such toxins, they should poison the normal, non-tuberculous as well as the tuberculous animal. But we have seen that extremely large doses are without untoward effect on the normal animals. If there are toxins, too, they should stimulate the animal organism to the production of antitoxin in amounts directly proportional to the quantity of tuberculo-protein introduced. As a matter of fact, no one has ever succeeded beyond dispute in producing an antitoxin to any derivative of the bacillus.

But how the picture changes when we consider animals that have already absorbed antigen! If an animal be infected experimentally it begins to react to tuberculin on or about the fifteenth day; in like manner, the non-tuberculous animal treated with protein will react to a second injection given about two weeks after the first. Both classes of animals will then be made violently ill by exceedingly small doses of the protein. What other explanation can we have but that in both there have been produced antibodies that react with antigen to set free a poison—the anaphylactic poison?

Nor do we have to assume that the tuberculous focus throws out toxins in order to explain the ordinary symptoms of the disease—the weakness, the anorexia, the tachycardia, the emaciation, the fever that are so often observed. Indeed it is more rational, in the light of our present knowledge, to ascribe these to the continual absorption of antigen by an already sensitized system. If an extract of the entire contents of several tubes of cultures of tubercle bacilli will not make a normal animal the least bit ill, why should the products of comparatively few bacilli, as many as would be enclosed in a tubercle the size of a walnut, bring about so severe an intoxication of an animal? It is hard to explain this on the toxin idea, but the conception of hypersensitiveness makes it much clearer.

The method of tuberculin therapy that is in most common use to-day is that of treating the individual with small doses and gradually raising them until the patient can tolerate quite large amounts without reaction. Every therapeutist comes to realize that there is no exact guide in this treatment and that his sole index in proceeding with tuberculin injections is the manner in which the patient responds to them. He finds that he cannot even predict which particular case will do well and which one will lose ground under treatment. He learns that so far as the results are concerned each individual patient is a law to himself; and that, if the latter holds his ground or improves, it is probably safe to continue with tuberculin, but if his health retrogrades it is better to discontinue it. Out of the tremendous mass of conflicting evidence that clinicians have given us, I think that several generalizations can fairly be made. These are that the chronic, afebrile case, who is in good condition and whose disease is not too far advanced, is the most favorable case for treatment; that such a patient's condition is often improved by tuberculin therapy after he has long ceased to show improvement under the other well-known methods of treatment; that those patients do best who can tolerate relatively large amounts of tuberculin, and conversely, those patients can best tolerate tuberculin who are most free from symptoms of their disease; and that in many cases the patient's tolerance to tuberculin is apparently greatly increased by spaced, gradually increasing doses of the antigen. And it is this latter principle—to diminish hypersensitiveness and its consequent intoxication—that has perhaps been the leading aim in therapy.

I therefore attempted to imitate the conditions of tuberculin therapy on non-tuberculous animals and to follow the results so far as sensitiveness was concerned. I wished to find out whether I could render them tolerant or immune to tuberculo-protein. In the experiment I used thirty-six normal guinea-pigs, which I divided into three sets of twelve each. Each set received spaced, gradually increasing amounts of water-extract at various intervals of time—one set every two days, another every four days, and a third every seven days. After they had reached fairly high dosage the animals were allowed to go without treatment for from seven to sixteen days, when they were given postorbital injections of extract-emulsion. The animals that were treated every two days began with 0.25 cc. W. E. subcutaneously and after thirty-nine days were getting 5 cc. intraperitoneally; those treated every four days began with 0.25 cc. subcutaneously and ended on the thirty-ninth day with 4 cc. intraperitoneally; those treated every seven days began with 0.25 cc. subcutaneously and ended on the thirty-ninth day with 4 cc. intraperitoneally. The first set received a total of 25 cc. W. E. in seventeen doses; the second, a total of 13 cc. in ten doses; the third, a total of 8 cc. in six doses. Seven days after treatment stopped, I tested several members of each series postorbitally, and again sixteen days afterwards. All of the animals except one were very sensitive to the ordinary toxic doses for sensitized guinea-pigs (0.75 cc. E. E.), while most of them reacted strongly to very small doses (0.15 cc. to 0.3 cc.). The general result was to indicate that after repeated doses sensitiveness was much greater than after single injections. There was no evidence that tuberculo-protein toleration could be obtained in non-tuberculous animals by repeated dosing,—there was, at least, no noticeable tolerance seven days after treatment stopped. A further endeavor was then made to discover whether there was any tolerance whatever for a very short time after regular treatment with the protein. I therefore repeated the experiment I have just cited, making the toxic, postorbital injection three days after the last subcutaneous treatment. I thus tested four animals and found them all sensitive, three of them fatally so. I have since treated many animals with more than a single sensitizing injection and have found that the best way to attain a very high degree of sensitiveness is to give them repeated injections of antigen—and that the larger the individual doses of antigen, the greater the degree of sensitiveness.

These experiments led to two conclusions; namely, first, once sensitiveness to tuberculo-protein is acquired by a non-tuberculous animal, the repeated absorption of protein tends only to increase this condition; and second, tolerance to tuberculo-protein cannot be attained in these animals by gradually raising the dosage or diminishing the intervals of time between doses. Other experiments, which I shall cite later, showed that perceptible sensitiveness persists for a long time after a single injection—often for more than a year—but that there is a tendency for it to diminish as time goes on unless it is renewed by further applications of antigen.

The results of the experiments which I have just described do not seem to square with the clinical idea of the method of gaining tolerance in the tuberculous, and it is this contradiction to which we must now address ourselves. As the first step, let us examine the nature of the different forms of sensitiveness as they exist in the tuberculous organism.

If we treat the skin with a derivative of the tubercle bacillus either by applying the latter to a break in the surface or injecting it between the layers of the skin, we find that some hours afterwards an inflammation will occur at the site of or in the immediate neighborhood of the injection. All of our present knowledge indicates that this inflammation is the ultimate result of an interaction between the injected antigen and antibodies that are present at the point of injection, and that

these antibodies are produced by the diseased organism in response to antigen that has arisen from the tuberculous focus. According to von Pirquet's view all of the cells of the body take part in this response to stimulation by antigen. At the Saranac laboratory we have never been able to elicit a skin reaction in guinea-pigs unless the animal has tubercles caused by living or dead bacilli; that is, a first injection of extracted protein will not sensitize to a later skin reaction. Moss, however, has found that individuals sensitized with horse serum acquire a skin sensitiveness to the serum. The possibility of sensitizing the skin by injecting an animal with pure tuberculo-protein is therefore not so remote if we once hit upon the proper conditions; and we are still working along this line.

If a small amount of tuberculo-protein enters the body subcutaneously or intraperitoneally or even intravenously (provided in the last instance that not too much anaphylactic antibody is present in the circulation) the tuberculous focus will suffer a reaction. This reaction also comes on several hours after the injection and expresses itself symptomatically by evidences of increased activity of the disease process, such as cough and expectoration in pulmonary tuberculosis, and anatomically by inflammation. As in the skin reactions, this inflammation is also most likely the end result of the union of antigen and anaphylactic antibody at the focus. Now the inflammation of the focus introduces a factor altogether different from any that can exist in the sensitized, non-tuberculous animal. By bringing about circulatory changes and softening the focus it renders the absorption of the focal material much easier than it was before. This focal material thrown rapidly into the circulation then reacts on an organism that has already been sensitized to it during the course of the disease and brings about an acute type of illness, the so-called general fever reaction, the manifestations of which are not at all unlike those met with in any acute exacerbation of the disease. Indeed, there is great similarity between what occurs during the general reaction and the course of events in ordinary acute tuberculosis. In both there are signs and symptoms of increased inflammatory focal changes: in both, the symptoms are very much alike. I have already said that the conception that the symptoms of tuberculosis are due to the absorption of focal material is more reasonable than the primary toxin idea; and it is evidence such as this that leads me to think that the general reaction is a phenomenon that depends upon what happens at the focus after a tuberculin injection. I am at the present time engaged in experiments in an attempt to discover what the sensitizing material of the focus is: whether it is the bacilli or the necrotic material or a substance that is formed by the effect of one acting on the other. My results are still very incomplete, but I may say here that I have not been able to confirm Kraus and Volk's observation that extracts of focal material are primarily toxic for guinea-pigs while those of normal organs are not.

Besides these three forms of reaction there is the so-called puncture reaction, which is essentially a local reaction and similar to the skin reactions, and the acute anaphylactic reaction, which we have already discussed.

Let us take such a tuberculous animal with local, focal and what I may call acute anaphylactic sensitiveness and wander into more or less hypothetical ground. If we should inject it subcutaneously with tuberculin we might get a puncture reaction and no other perceptible response to the injection. Here I should say that at the point of injection there was enough anaphylactic antibody present to bind all or most of the antigen, so that if there was a residuum of the latter it was not enough to combine with sufficient antibody to cause reaction elsewhere. Most of the protein would be neutralized, as it were, at the surface of the body. If, however, the peripheral antibody were absent, or small in amount, or if the doses were considerably larger than what the peripheral antibody could take care of, the antigen would get into the circulation. Here it would meet with circulating anaphylactic antibody if the latter were present. The acute anaphylactic shock would not result, however, because on account of slow absorption the units of antigen would be picked up one by one, and we have already seen that the fulminant symptoms are only brought on when we introduce the antigen in such a manner that it gets into the circulation very rapidly. Whatever antigen is bound by circulating anaphylactic antibody becomes incapable of reacting with the anaphylactic antibody in the focus and thus the focus is protected to a degree that is directly proportional to the amount of circulating antibody that exists at any one time. We should have here a very unusual condition. The circulating anaphylactic antibody would be a great menace to an animal if the antigen were injected intravenously, but it is just as great a bulwark against focal and general reactions if the antigen is so injected that absorption is slow. With the combination proceeding gradually unit by unit all the circulating antibody is used up without the production of symptoms, and the only way that the focus will be affected will be by the unbound residual antigen. Thus we should have two zones of defense, which I may call peripheral and circulating zones; and the contingency of a focal reaction would depend upon quantitative relations between peripheral and circulating antibody and antigen, as well as upon time relations that would govern the binding of the two opposing substances, which in turn would depend largely upon the method of injection and the degree of solubility of the antigen that is employed. I have approached this matter of absorption experimentally in the following manner. I first injected sensitized, non-tuberculous guinea-pigs with very large doses of extract-emulsion intraperitoneally and followed this up some time later with a post-orbital injection. When the second injection had been given some time (one-half to one hour and a half) after the first injection it very often failed to bring on the anaphylactic symptoms, showing that the circulating antibody had disappeared. These observations have been sufficiently controlled to be of some value, and I have experiments now under way in which I am trying to establish the time relations more definitely.

But there is a third defensive zone with which we have to do. This is of a mechanical nature and is comprised in the extent of fibrosis that any given tuberculous focus has undergone.

It seems to me that if a tubercle is well invested by fibrous tissue it can stand assaults by tuberculin much more successfully than if no such protection exists. As healing occurs, and more and more scarring takes place, and the tubercle becomes less and less vascular, it certainly must happen that less extraneous matter can be carried into the tubercle. This factor must surely be of prime importance in many cases. We know that as a general thing the longer a given case has been healed and the more completely a lesion has been closed in, the larger our dose of tuberculin must be to induce reactions. There are some exceptions to this rule but I believe the generalization can safely be made. Now the non-tuberculous animal, once sensitized, gradually loses this condition unless we inject more antigen—in other words, its circulating antibody diminishes in amount. The case that has been healed for a long time would so far be comparable to the former. We should assume, therefore, that it had less circulating anaphylactic antibody to protect its old focus. The tubercle, however, has a more or less efficient protection of its own, namely, its fibrous capsule.

We now begin tuberculin therapy with a tuberculous animal, such as we have been considering. If the tissues of the tuberculous animal respond to injections of tuberculo-protein like those of the non-tuberculous animal—and I see no reason why they should not—they will gradually heap up anaphylactic antibody in the circulation. If there is very little of this antibody to begin with, or if the focus is naked, as it were, with but slight fibrosis, we have manifestly a more delicate task ahead of us than if the reverse is the case. We can conceive of several possible conditions of the patient at the time he starts treatment. He may have much circulating antibody with a well invested tuberculosis, or much circulating antibody with more or less softened tubercles, or little circulating antibody combined with either focal condition. It is therefore not hard to foresee how every case may respond in its own way to tuberculin. But if we have a favorable case to begin with, the tendency would be, as stated, to stimulate the organism to the production of circulating antibody, which, as we have assumed, protects the focus against subsequent doses of antigen and is one of the factors in the so-called acquired tolerance after treatment. I now have experiments under way to test the validity of this hypothesis. By repeated doses of antigen I sensitized normal animals to a high degree. I have lately rendered these sensitive animals tuberculous, together with normal, non-sensitive control animals. In a couple of months, after the disease is well established, I shall give both sets lethal doses of tuberculo-protein subcutaneously or intraperitoneally. If my hypothesis is correct I shall expect to find the initially non-sensitive animals reacting focally to smaller doses than those that were sensitized before inoculation.

The other factor in the production of tolerance in therapy would enter in the direct action of the tuberculo-protein on the focus itself. Since we know that tuberculo-protein inflames the tubercle, it is more than likely that the end result of every dose that reaches the tubercle, no matter how small, is to bring about changes that may vary from the most transient hyperæmia to the most intense inflammation of the focus. It seems quite reasonable that slight inflammations could be of real service in assisting the healing process of the tubercle and many clinical observations, which it is unnecessary to go into here, support this view. Certainly by the increased stimulation of fibrous reaction the scarring process would be hastened. The amount of tuberculo-protein which would react with the focus would depend upon the conditions that I have already mentioned. With a good defense of circulating antibody the focus would pick out only the residual tuberculo-protein and would be saved from the dangers of too violent a reaction—the excessive inflammation and a dissemination of the disease that might result were the tubercle subjected to all the protein of a given dose. Hyperæmia and inflammations during the course of treatment might be so slight as to be imperceptible, yet their end effects—the resultant focal sclerosis—might be marked. And as the focus gradually becomes closed in during the healing process, the third zone of defense that we have considered would become strengthened and assist appreciably in producing tolerance to the antigen.

I think that you have already become impressed by the fact that the problems of the tuberculin reaction and of tuberculin therapy are extremely complicated ones and that any adequate theories of them must take into consideration a great many factors. In the above recital I have tried to do no more than elaborate a working hypothesis that is based on well authenticated clinical observations and on experimental work that has been carried on at the Saranac laboratory. I have tried to separate what has been demonstrated from what remains yet to be proved before these conceptions can be lifted from the realms of hypothesis to those of theory. And if I have been successful in suggesting fruitful paths of experimentation I shall be more than satisfied.

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A problem of the first importance is what relation the hypersensitiveness to the products of a given microörganism bears to susceptibility to infection from it. It has long been known that during the course of an infection the diseased individual becomes sensitive to the organic products of the infecting agent, but thus far we have had practically no experimental information concerning the part that the sensitive state plays in the defensive processes of the organism against bacillary implantation. It was our problem, therefore, to discover whether the tuberculo-protein sensitized animal acquired increased or lowered resistance to infection by the tubercle bacillus.

I accordingly undertook experiments in which I aimed, first, to sensitize normal animals; next, to inoculate these together with normal, non-sensitive controls; and finally, to sacrifice the whole number after the tuberculosis was well established and compare the progress of the disease in the initially sensitive animals with that in the control animals. The animals that I sensitized before inoculation comprised forty-two guinea-pigs which were divided into four sets. The members

of set A received a subcutaneous or intraperitoneal injection of water-extract every two days over a period of thirty-nine days in which they got a total of 25 cc. Those of set B were similarly treated every four days and received a total of 13 cc. in the thirty-nine days. Each animal of set C, treated every seven days, had in all 8 cc. during the thirty-nine days. The individuals of set D were given only one sensitizing injection,—1.25 cc. of water-extract intraperitoneally. There were originally twelve animals in set A, twelve in set B, twelve in set C and six in set D. Sixteen days after the preliminary treatment of sets A, B and C ended, I tested individuals from each lot and found that a high degree of sensitiveness to extract-emulsion existed in all of the tested animals. At the same time I gave two members of each set A, B and C sub-lethal doses. These last named animals all showed severe anaphylactic symptoms but did not die. I did not test the animals of set D, because I had sensitized animals in a similar manner many times previously and had always found them hypersensitive.

I was now ready for my inoculation. For this purpose I took six animals, that were sensitive but had not been tested, from each of the sets, A, B, C, and D; two from each of the sets A, B, and C that had received a toxic postorbital injection an hour before, had shown symptoms but had to a certain extent recovered from them; and six normal non-sensitized animals as controls. I thus had six different lots for inoculation, made up as follows: Lot 1, six guinea-pigs that had been sensitized, then given sub-lethal toxic injections, and were more or less intoxicated at the time of inoculation. These animals were in a refractory condition to further immediate injections of protein, since their first non-lethal toxic injection protected them against a second. Lot 2, six guinea-pigs, sensitized by treatment every two days, and not given toxic injections. Lot 3, six guinea-pigs, sensitized by treatment every four days, not given toxic injections. Lot 4, six guinea-pigs, sensitized by treatment every seven days, not given toxic injections. Lot 5, six guinea-pigs, sensitized by one injection thirty-seven days before inoculation. Lot 6, six normal untreated guinea-pigs.

I injected each of the above animals in the same spot subcutaneously in the right groin with one-tenth of a cubic centimeter of an emulsion of human tubercle bacilli prepared as follows: Several spadefuls of a beef serum culture were rubbed up in a mortar with a minimal amount of physiological salt solution until the mixture was a fairly homogeneous paste. This was then diluted and centrifugalized several times. The supernatant emulsion was pipetted off, further diluted and again centrifugalized. The resultant supernatant emulsion was then filtered several times through cotton. This filtrate was the final emulsion that was used for inoculation. Examination of the filtrate showed no clumps and an average of but one tubercle bacillus to every one or two fields of the oil immersion lens. Before inoculating each animal the beaker containing the stock emulsion was given the same number of whirls to ensure an even mixture and but one-tenth of a cubic centimeter was withdrawn at any one time.

Sixty-two days afterwards the animals were killed and their condition noted. The results of the inoculation can be summarized as follows:[2]

*Refractory Animals, 5 guinea pigs.*—Total weight at time of inoculation, 1795 grams; average weight, 369 grams; total weight, 62 days later, 2200 grams; average weight, 440 grams; average net gain, 81 grams. None pregnant. There is advanced tuberculosis in all. The lungs and spleen are involved in every animal, and the liver in two.

*Sensitized Animals, treated every 2 days, 6 guinea pigs.*—Total weight at time of inoculation, 2265 grams; average weight, 377 grams; total weight, 62 days later, 2690 grams; average weight, 448 grams; average net gain, 71 grams. Three were pregnant. The disease has reached the spleen in only one of the six and has advanced no further in any.

*Sensitized Animals, treated every four days, 6 guinea pigs.*—Total weight at time of inoculation, 2100 grams; average weight, 350 grams; total weight, 62 days later, 2650 grams; average weight, 442 grams; average net gain, 92 grams. Two were pregnant, one of which littered 21 days after inoculation. The spleen is involved in three of the six guinea pigs. The process has affected other viscera in only one which was pregnant and in which the lungs were diseased.

*Sensitized Animals, treated every 7 days, 6 guinea pigs.*—Total weight at time of inoculation, 2185 grams; average weight, 364 grams; total weight, 62 days later, 2950 grams; average weight, 492 grams; average net gain, 128 grams. Two were pregnant. The spleen was involved in three, and the lungs in two of these three.

*Sensitized Animals, given only one dose, 6 guinea pigs.*—Total weight at time of inoculation, 2140 grams; average weight, 357 grams; total weight, 62 days later, 2680 grams; average weight, 447 grams; average net gain, 90 grams. One was pregnant. The spleen was diseased in every animal and the lungs in two.

*Control, Non-sensitized Animals, 6 guinea pigs, 5 autopsied.*—Total weight at time of inoculation, 2180 grams; average weight, 436 grams; total weight 62 days later, 2830 grams; average weight, 566 grams; average net gain, 130 grams. None were pregnant. The spleen was involved in four of the five animals and the lungs in two.

So far as known, all of the pregnant animals became pregnant after inoculation. Macroscopic tuberculosis is meant in the above descriptions when an organ is said to be involved.

The refractory animals suffered most. The disease was pretty well disseminated in all of these and they exhibited far more tuberculosis than any of the animals that had not been intoxicated and than any of the controls. It will be remembered that these refractory animals were given toxic injections about an hour before inoculation. The widespread extent of the process was very likely due to the fact that the bacilli were implanted in animals that were still more or less poisoned at the moment of infection. And it would appear that the animal organism is less resistant to infection than normal during the period that it is undergoing symptoms of anaphylaxis or before it has completely recovered from their effects. Whether this event is specific or whether toxicity from one bacterio-protein would fail to render an animal more susceptible to infection by another microörganism is a matter that has not yet been determined. We know that some infectious diseases undoubtedly increase the susceptibility to tuberculosis: or, at any rate, that tuberculosis often actively

[2] Details of these experiments are to be found in an article in the Journal of Medical Research, 1911, XXIV, 2, 361.

appears during or after the course of another disease. It would be interesting and important to find out whether this diminished resistance to tuberculosis is due to the intoxication that is brought about by hypersensitiveness to another bacterio-protein, and I am now trying to solve this problem by experimentation.

The animals that were sensitized in various ways all became diseased. As a general thing, we may say that the more protein the animal received during preliminary treatment, the less the resultant infection. So far as I could tell from the toxic symptoms of the tested animals, there was very little difference in the average degree of sensitization between members of the several sets. Nevertheless, the reaction is a gross one and the mere fact that animals from three different series reacted fatally does not imply that their degree of sensitization is the same. You will easily see that if one thousand antibody units, say, or 0.3 cc. of antigen, were enough to produce death in a given guinea-pig, then ten thousand antibody units or 0.5 cc. of antigen would bring about the same result in another guinea-pig. Both die but are not equally sensitive, for guinea-pig B would have ten times as much antibody as guinea-pig A. We can, however, determine the degree of sensitiveness by graduating the dose of antigen which would be the constant. The guinea-pig would be the variable and its symptoms in response to a fixed, moderate dose of antigen would determine its sensitiveness. Unfortunately in these experiments I did not have enough animals to determine these variations. Since the preparation of an article that has lately been published and in which I assumed that the animals of the different series were probably equally sensitive, I have had occasion to deduce from a number of experiments that sensitiveness does fluctuate—tending to die out, though very slowly indeed, if the antigen is not renewed and being markedly increased by repeated injections. I am glad therefore to take this opportunity to qualify my former assumption and point out its fallacy.

However, it is very likely that the differences of tuberculous involvement were altogether independent of any degree of raised or lowered resistance conferred by the sensitive state, but that they were due to a heightened immunity that followed the protein injections and was brought about by other unknown factors. The changes that occurred in those guinea-pigs that had only one sensitizing dose support this view. These were inoculated thirty-seven days after sensitization. Experiments covering a period of two years have shown us that thirty-seven days after an intraperitoneal injection of 1.25 cc. of water extract, a guinea-pig is almost invariably fatally sensitive. So we are pretty safe in assuming that at the time of inoculation these were highly sensitive. Yet these "one dose" animals suffered in just about the same measure as did the controls. The one dose of water-extract was not sufficient to make them immune to infection. But it was enough to sensitize them, and while sensitive they contracted about as much tuberculosis as did the normal animals. This evidence would therefore strengthen the opinion that sensitization to a bacterio-protein of itself does not convey immunity to infection, and also that hypersensitiveness and increased resistance are probably two different phenomena. The experiment also shows definitely that they can exist side by side in the same animal.

But we must remember that in our experiments we have very likely introduced into the animal body relatively large amounts of the infecting agent compared to what is taken in under natural conditions of infection. It is conceivable that the degree of sensitiveness that would express itself by fatal anaphylaxis might confer upon the animal the capacity to get rid of ten or fifty or one hundred tubercle bacilli, numbers that probably represent the amount of infecting material that is naturally introduced more nearly than the number contained in our inoculated emulsion. The question is certainly an open one and the point must be emphasized. At the Saranac laboratory we are now employing the more exact method of Barber in our inoculation of sensitized animals. This as you know consists in counting out the living microörganisms one by one, and thus enables us to infect with any desired number from one up. By the use of this method we hope to determine definitely whether sensitization carries with it any increased resistance to infection.

* * * * * * * * * *

There remains one phase of our work on sensitization which I should like to bring before you. This is the matter of the inheritance of tuberculo-protein sensitiveness. Most of you know that Rosenau and Anderson first demonstrated that sensitiveness to horse serum was transmitted from the female guinea-pig to its offspring and that the male parent has no influence in this transmission. Their work has been confirmed by Gay and Southard, Lewis, and Otto. Schenck has brought forward some evidence that serum sensitiveness may also to a slight degree be handed down from the male parent. In his work on tuberculo-protein anaphylaxis, Baldwin found that the law of maternal transmission also held good with this bacillary derivative. Guinea-pigs, the offspring of actively sensitized mothers, reacted when one or two months old.

Although Baldwin was unable to show in a few animals that the tuberculous mother, which has not been treated with tuberculo-protein, transmits sensitiveness to her young, the chances are in favor of the possibility that in some instances she actually does so. You will remember that a certain proportion of tuberculous animals react to postorbital injection of bacillary protein with acute anaphylaxis in exactly the same manner as non-tuberculous sensitized ones; and that the similarity of the reaction compelled the conclusion that the reaction is in both instances the resultant of the same factors; namely, an interaction of antigen and circulating antibody. Since therefore the non-treated tuberculous animal which will react and the treated, hypersensitive animal are in exactly the same anaphylactic condition which results from the same fundamental cause, it would seem certain that in some cases tuberculous females would bear offspring that are perceptibly sensitive. This would necessarily follow in an animal that would conceive and carry young to term during a period in which she herself had considerable circulating antibody and was very sensitive. Thus far in our laboratory we have come across no

sensitive offspring of tuberculous mothers, but we are still testing all such young, in the belief that sooner or later we shall encounter a sensitive guinea-pig.

There is probably no other disease that has occasioned so much discussion in regard to hereditary influences as tuberculosis, and any addition to our knowledge on this subject further than mere surmise is manifestly important. Baldwin's discovery of the maternal transmission of tuberculo-protein hypersensitiveness is, I believe, the first satisfactory evidence of the inheritance of any specific property in relation to tuberculosis. While we do not yet know what part the hypersensitive condition to bacterial products plays in immunity—whether it protects against natural infection or lays the animal organism more open to invasion—it seemed worth while to try to find out how constantly the tuberculo-protein sensitized mother will transmit the condition, and how long the animal that is born sensitive will retain the anaphylactic state. Because, too, of the logical inference, as outlined above, that under certain conditions tuberculous mothers must confer a sensitiveness on their offspring that may be perceptible or imperceptible, I thought it well to continue Baldwin's experiments.

I tested a total of sixty-one young, giving them single post-orbital or intravenous injections of extract-emulsion. All were the offspring of presumably sensitive mothers which were not tuberculous. When they were tested they varied greatly in age as well as in the time that had elapsed between the mother's last dose of antigen and the birth of the different animals. I shall not burden you with the details of the experiments, which may be found in another publication, but shall merely state their results, which may be summed up as follows:

1. The possibility of maternal inheritance is more or less irregular and inconstant.

2. It depends largely on the degree of sensitiveness of the mother at the time of pregnancy.

3. The degree of sensitiveness of the offspring as a rule varies directly with that of the mother.

4. The mother's sensitiveness is heightened by repeated applications of antigen, and, conversely, tends to die out with time unless renewed by subsequent injections.

5. The degree of sensitiveness of the offspring accordingly varies, depending on the time that has elapsed between the mother's last dose and the birth of the offspring.

6. The degree of sensitiveness that an animal inherits tends to diminish as it increases in age and size.

7. Guinea-pigs have been born sensitive 379 days after their mother's last injection of antigen. They have also remained sensitive as long as 404 days after birth, although they had never been tested before.

8. Animals of the same litter may vary greatly in the degree of sensitiveness which they inherit.

9. The fact that a number of animals were born sensitive over a hundred days, and several 379 days, after their mothers' last injection, is direct evidence that the transmission may be one of anaphylactic antibody and not of the antigen.

10. The transmission by inheritance is probably always or mainly one of antibodies.

11. Hypersensitiveness to tuberculo-protein is most likely never handed down to the third generation. This conclusion is based on tests on nine animals, the grandchildren of sensitized females through the female line.

All this evidence concerning inheritance will probably be of more tangible value after we find out what rôle hypersensitiveness plays in infection. Meanwhile it furnishes encouragement for more experimentation in this direction.

In the above recital of experimental work performed and in the discussion of where such investigations lead, I have done little more than state the problem of tuberculo-protein hypersensitiveness in its relation to several of the phases of tuberculous infection. I thank you heartily for the forbearance with which you have followed me, and trust that the future will bring forth still more fruitful results than the past.

# VARIATIONS IN THE LEUCOCYTE COUNT IN NORMAL RABBITS, IN RABBITS FOLLOWING THE INJECTION OF NORMAL HORSE SERUM, AND DURING A CUTANEOUS ANAPHYLACTIC REACTION.

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The observations to be reported in this paper were made in connection with some recent work of Knox, Moss and Brown (The Journal of Experimental Medicine, 1910, XII, No. 4) on a local anaphylactic reaction in rabbits. Healthy rabbits were sensitized by the intravenous injection of normal horse serum in doses of 0.1 cc. to 5 cc. After an interval of 12 days or longer their sensitiveness was tested by an intradermal injection of 0.01 cc. normal horse serum given in the skin of the abdomen. A positive reaction consists of localized inflammation and swelling, varying from 0.5 cm. to 2 cm. in diameter which appears within 24 hours and usually subsides in three or four days.

In connection with this work we attempted to determine if the total or differential leucocyte counts underwent any characteristic changes either following the sensitizing injection or during the local anaphylactic reaction produced by the intradermal injection of 0.01 cc. horse serum.

A considerable number of observations have been published on changes in the leucocytes following the injection of various antitoxic sera and during the anaphylactic reaction in human beings, a very good summary of which appears by Bianco Bienenfeld (Jahrbuch für Kinderheilkunde und Physiche Erziehung, N. F., Bd. LXV, 1907). Bienenfeld's personal observations showed a leucopenia, immediately following the injection of large doses of serum, followed by a leucocytosis, which, in most cases of the serum disease, in turn gave place to a recurrence of leucopenia. The leucopenia is ascribed to negative chemotaxis and is characterized by a diminution of the polymorphonuclear neutrophiles. The nongranular cells seem much more resistant to these changes than do the granular cells, since they undergo much less diminution. The eosinophiles are not affected. Bienenfeld states that toward the end of the leucopenia there appear many large mononuclear and transitional cells. These are ascribed to regenerative processes in the blood and lead to the belief that during the serum disease there is destruction of leucocytes followed by regeneration; however, owing to lack of more positive proof, the author leaves the question open as to the cause of the leucopenia, whether it is due to non-functionating of the blood-forming organs or to a destruction of leucocytes.

Hamburger and v. Reuss (Ueber die Wirkung artfremden genuinen Eiweisses auf die Leukozyten. Zeitschrift für Biologie, 1910, XLVII, 24) in a large series of experiments carried out on rabbits found that the intravenous injection of foreign proteids is followed in about five minutes by a marked diminution in the number of leucocytes. An exception to this rule was found in the case of horse serum which caused practically the same result as the injection of homologous (rabbit) serum, namely, a hyperleucocytosis. The same result, hyperleucocytosis, followed the injection of normal salt solution and indeed simple venepuncture without any injection. The authors consider the hypoleucocytosis following the injection of foreign proteid, the result of a toxic action.

Aschenheim (Ueber Schwankungen der Leukocytenzahle nach Traumen und Injektionen. Zeitschrift für Biologie, 1910, LI, 385) injected a series of rabbits with homologous and heterologous serum and in opposition to Hamburger and v. Reuss observed hypoleucocytosis following the homologous, as well as the heterologous serum. Simple venepuncture, without injection of any kind, was followed by hyperleucocytosis. Aschenheim was unable to refer the effect on the leucocyte count of simple venepuncture to reflex vasomotor changes due to painful stimuli; since anesthesia did not prevent the occurrence of hyperleucocytosis. He admits, however, that his experiments were not conclusive since the sympathetic system may not have been inactive under anesthesia.

Subcutaneous injections into human beings of human serum and serum from a variety of animals, produced changes in the leucocyte count which followed no definite rule.

In the light of our own experience, which did not come until after many counts had been made, we feel that in none of the work which we have seen reported, has enough care been bestowed upon the establishment of the variations in the normal leucocyte counts in rabbits. It was not until after we had obtained many confusing results that we devoted our attention seriously to this point.

Before giving the results of our counts a brief description of the conditions under which the work was carried out will be given.

Young adult rabbits about a year old and of approximately the same size and weight were selected from a large number of country-raised rabbits. The males were separated from the females throughout the experiments and no pregnant females were used.

In the first series tested, as many as four or five rabbits were kept in a three- by four-foot cage, but in the subsequent series each rabbit was kept in a separate cage in order to prevent fighting, which might result in injuries or infections and thus influence the leucocytes.

All rabbits were fed once a day at approximately the same hour, 10 a. m. The food consisted of greens, grain and hay in such quantity that all would usually be consumed in four to six hours after feeding.

The blood for counting was taken from an ear vein and to insure a free flow of blood, after the ear was shaved, cleaned with alcohol and dried, a test tube containing warm water was placed beneath the ear; this caused a prompt dilatation of the veins and free flow of blood. As soon as the pipette for counting had been filled, smears for the differential counts were made.

Thoma-Zeiss blood counting apparatus was used; the pipettes were of the large, 1-20 dilution variety, the diluent was prepared fresh each day by adding four drops of glacial acetic acid to 40 cc. distilled water. In all cases two slides of five square millimeters each were counted in order to determine the total number of leucocytes.

The films for the differential counts were stained with Hastings' stain and a total of 200 cells counted for each differential. Duplicate counts made in a number of instances showed that this number of cells gave percentages which agreed quite closely with those given by counting 400 cells or more.

Since the value of this work depends entirely upon the accuracy of the counts we have attempted to get some idea of the percentage error in our counts. To this end we made duplicate determinations at frequent intervals throughout the work. A free flow of blood having been obtained from the ear vein, two pipettes were filled in rapid succession and two slides of five square millimeters each counted from each pipette. The average percentage variation of the two counts from the mean of the two counts in 48 such duplicate determinations was 7.5 per cent.

We further tried to get an idea of the accuracy of the counts by determining the percentage variation of each slide from the average of the two slides. In 174 such determinations the average percentage variation between slides was 3.9 per cent.

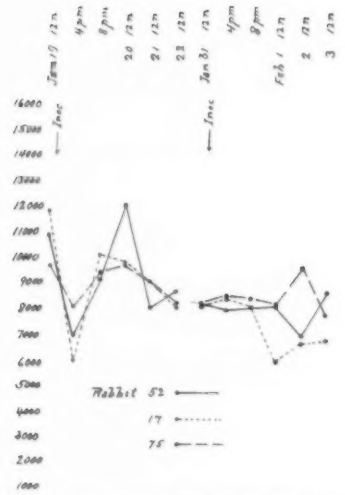


CHART I.—TOTAL LEUCOCYTE COUNTS.

Jan. 19 injected normal horse serum intravenously as follows: Rabbit No. 52, 0.1 cc.; Rabbit No. 17, 1 cc.; Rabbit No. 75, 5 cc. Jan. 31 injected 0.01 cc. normal horse serum intradermally in all three rabbits.

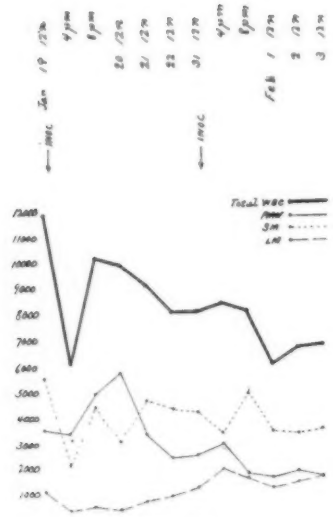


CHART III.

Rabbit No. 17 showing total leucocyte count and absolute values for polymorphonuclear, large and small mononuclear leucocytes.

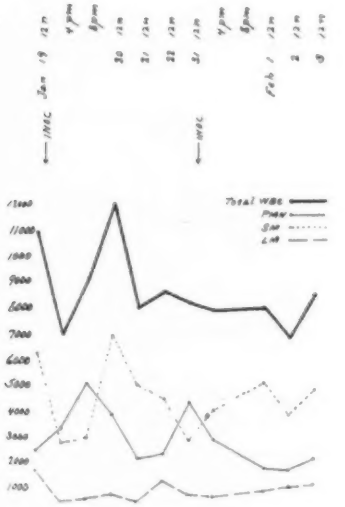


CHART II.

Rabbit No. 52 showing total leucocyte count and absolute values for polymorphonuclear, large and small mononuclear leucocytes.

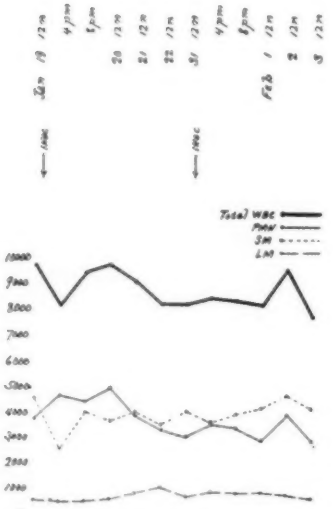


CHART IV.

Rabbit No. 75 showing total leucocyte count and absolute values for polymorphonuclear, large and small mononuclear leucocytes.

On two occasions we filled three pipettes in rapid succession from the same rabbit and twice we filled five pipettes. The results of the counts made from the separate pipettes are tabulated below:

| | Rabbit No. 23, March 14, 1910 | Rabbit A | Rabbit No. 99, May 19, 1910 | Rabbit No. 99, May 20, 1910 |
|---|---|---|---|---|
| 1st pipette ..... | 8670 | 7360 | 4100 | 3540 |
| 2d pipette ..... | 8660 | 7880 | 4020 | 4060 |
| 3d pipette ..... | 8470 | 7720 | 3920 | 3560 |
| 4th pipette ..... | | | 4220 | 4060 |
| 5th pipette ..... | | | 3780 | 3700 |
| Average ..... | 8600 | 7653 | 4008 | 3784 |
| Greatest variation from average.... | 1.5% | 3.8% | 5.4% | 7.3% |

The above percentages represent all the data we have on the accuracy of the counts and we feel they indicate that our results may be taken as trustworthy.

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Rabbit No. 52. January 19, 1910, injected 0.1 cc. normal horse serum intravenously. January 31, 1910, cutaneous anaphylactic test, 0.01 cc. normal horse serum intradermally. Local reaction negative.

| | Total Leucocytes | Pmn* % | Pmn* Total | Lm % | Lm Total | Sm % | Sm Total | Eos % | Eos Total | Mst % | Mst Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Before injection... | 10900 | 22.5 | 2452 | 15.0 | 1635 | 57.5 | 6268 | 3.5 | 381 | 1.5 | 164 |
| 4 hours after.... | 7000 | 47.4 | 3318 | 6.6 | 462 | 39.4 | 2758 | 4.0 | 280 | 2.4 | 168 |
| 8 " " .... | 9100 | 55.6 | 5060 | 6.3 | 574 | 32.6 | 2966 | 1.6 | 146 | 3.6 | 328 |
| 24 " " .... | 12000 | 32.3 | 3876 | 6.0 | 720 | 57.6 | 6912 | 1.3 | 156 | 2.6 | 312 |
| 48 " " .... | 8000 | 27.0 | 2160 | 5.5 | 440 | 63.0 | 5040 | 2.5 | 200 | 2.0 | 160 |
| 72 " " .... | 8600 | 27.6 | 2373 | 14.3 | 1230 | 52.0 | 4477 | 4.0 | 344 | 2.0 | 174 |
| Before test..... | 8200 | 52.3 | 4287 | 9.4 | 771 | 35.0 | 2870 | 1.5 | 124 | 1.6 | 132 |
| 4 hours after.... | 7900 | 36.6 | 2892 | 8.6 | 680 | 51.3 | 4053 | 2.6 | 206 | 0.6 | 48 |
| 8 " " .... | Lost | 42.8 | .. | 9.2 | .. | 45.2 | .. | 1.6 | .. | 1.2 | .. |
| 24 " " .... | 8000 | 22.0 | 1760 | 11.0 | 880 | 64.3 | 5144 | 0.3 | 24 | 2.3 | 184 |
| 48 " " .... | 6900 | 25.0 | 1725 | 15.0 | 1035 | 55.5 | 3829 | 1.5 | 104 | 3.0 | 207 |
| 72 " " .... | 8500 | 25.0 | 2125 | 14.0 | 1190 | 57.0 | 4845 | 4.0 | 340 | .. | .. |

Rabbit No. 17. January 19, 1910, injected 1.0 cc. normal horse serum intravenously. January 31, 1910, cutaneous anaphylactic test, 0.01 cc. normal horse serum intradermally. Local reaction doubtful.

| | Total Leucocytes | Pmn % | Pmn Total | Lm % | Lm Total | Sm % | Sm Total | Eos % | Eos Total | Mst % | Mst Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Before injection... | 11800 | 29.8 | 3517 | 9.0 | 1062 | 48.9 | 5535 | 9.4 | 1110 | 4.7 | 555 |
| 4 hours after.... | 6100 | 54.6 | 3331 | 5.0 | 305 | 34.0 | 2074 | 5.3 | 324 | 1.0 | 61 |
| 8 " " .... | 10100 | 48.3 | 4879 | 4.6 | 465 | 43.0 | 4343 | 3.0 | 303 | 1.0 | 101 |
| 24 " " .... | 9800 | 58.3 | 5714 | 3.6 | 353 | 30.6 | 2999 | 3.6 | 353 | 3.6 | 353 |
| 48 " " .... | 9000 | 36.3 | 3267 | 7.6 | 684 | 51.0 | 4590 | 4.0 | 360 | 1.0 | 90 |
| 72 " " .... | 8000 | 29.6 | 2368 | 10.4 | 832 | 53.2 | 4256 | 3.2 | 256 | 3.6 | 288 |
| Before test..... | 8000 | 30.6 | 2448 | 14.3 | 1144 | 51.6 | 4128 | 1.3 | 104 | 2.0 | 160 |
| 4 hours after.... | 8300 | 34.3 | 2847 | 21.3 | 1770 | 39.0 | 3237 | 2.3 | 191 | 3.0 | 249 |
| 8 " " .... | 8000 | 20.0 | 1600 | 18.0 | 1440 | 60.4 | 4832 | 0.8 | 64 | 0.8 | 64 |
| 24 " " .... | 6000 | 24.0 | 1440 | 18.0 | 1080 | 55.2 | 3312 | 0.8 | 48 | 2.0 | 120 |
| 48 " " .... | 6800 | 25.5 | 1683 | 19.5 | 1287 | 49.5 | 3267 | 3.0 | 198 | 2.5 | 165 |
| 72 " " .... | 6700 | 21.5 | 1440 | 22.5 | 1508 | 51.0 | 3417 | 1.5 | 100 | 3.5 | 235 |

Rabbit No. 75. January 19, 1910, injected 5.0 cc. normal horse serum intravenously. January 31, 1910, cutaneous anaphylactic test, 0.01 cc. normal horse serum intradermally. Local reaction positive.

| | Total Leucocytes | Pmn % | Pmn Total | Lm % | Lm Total | Sm % | Sm Total | Eos % | Eos Total | Mst % | Mst Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Before injection... | 9700 | 38.7 | 3753 | 5.7 | 552 | 46.7 | 4529 | 7.0 | 679 | 1.8 | 174 |
| 4 hours after.... | 8100 | 57.3 | 4641 | 6.0 | 486 | 32.0 | 2592 | 4.0 | 324 | 0.7 | 56 |
| 8 " " .... | 9400 | 46.6 | 4380 | 5.6 | 526 | 42.3 | 3976 | 4.6 | 432 | 0.7 | 65 |
| 24 " " .... | 9700 | 51.0 | 4947 | 6.0 | 583 | 38.2 | 3705 | 3.2 | 310 | 1.5 | 145 |
| 48 " " .... | 9000 | 42.6 | 3834 | 9.0 | 810 | 44.6 | 4014 | 3.0 | 270 | 0.6 | 54 |
| 72 " " .... | 8200 | 40.0 | 3280 | 13.0 | 1066 | 43.0 | 3526 | 2.0 | 164 | 2.0 | 164 |
| Before test..... | 8200 | 37.2 | 3050 | 8.8 | 722 | 49.2 | 4035 | 1.6 | 131 | 3.2 | 262 |
| 4 hours after.... | 8400 | 42.4 | 3562 | 10.8 | 907 | 43.2 | 3629 | 0.8 | 67 | 2.8 | 235 |
| 8 " " .... | 8300 | 40.8 | 3386 | 10.0 | 830 | 46.8 | 3884 | 1.6 | 133 | 0.8 | 66 |
| 24 " " .... | 8100 | 35.6 | 2884 | 10.4 | 842 | 52.0 | 4212 | 0.4 | 32 | 1.6 | 130 |
| 48 " " .... | 9500 | 41.0 | 3895 | 8.0 | 760 | 49.0 | 4655 | .. | .. | 2.0 | 190 |
| 72 " " .... | 7700 | 36.0 | 2772 | 7.5 | 578 | 53.3 | 4104 | 1.3 | 100 | 1.9 | 147 |

The variations which the leucocytes have undergone are more readily seen if the above figures are plotted out in the form of curves. Chart I shows the total leucocyte count for the three rabbits. Four hours after the intravenous inoculation of normal horse serum the leucocyte count had fallen in all the rabbits. The greatest drop is in rabbit No. 17, which received 1 cc. serum; the next greatest drop is in No. 52 which received 0.1 cc. serum; while the leucocytes of No. 75, which received 5 cc. serum, showed the smallest drop. Eight hours after the inoculation the leucocytes of all three rabbits had risen but not to their original levels. At the end of 24 hours the leucocytes of No. 52 slightly exceeded their original level and from this point on to the end of 72 hours all of the curves fell.

Following the intradermal injection of 0.01 cc. serum there was practically no change in the total leucocyte counts at the end of four and eight hours, in any of the rabbits. At the end of 48 hours the leucocytes of No. 75 were slightly increased. This rabbit was the only one of the series which gave a well marked positive reaction.

Charts II, III and IV show the total leucocyte count, the total number of polymorphonuclears, small and large mononuclears plotted out separately for each rabbit. It is evident from these three charts that the low leucocyte counts, four hours after inoculation, are due to a fall in the small mononuclears; this fall being great enough to bring the total count down in spite of a slight increase in the polymorphonuclears in the case of Nos. 52 and 75. In the case of No. 52, eight hours after inoculation the increase in the total count is due largely to an increase in the polymorphonuclears, while the still further increase in the total count at the end of 24 hours is due to a recovery of the small mononuclears which elements carry the curve to its high point in spite of a falling off of the polymorphonuclears at this time. There seems to be nothing noteworthy about the differential counts following the intradermal inoculation.

SECOND SERIES.

| Rabbit No. 15 1910 | Total Leucocytes | Pmn % | Pmn Total | Lm % | Lm Total | Sm % | Sm Total | Eos % | Eos Total | Mst % | Mst Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| March 2, 9 a. m. | 10195 | 36.0 | 3670 | 8.5 | 866 | 52.5 | 5352 | 1.0 | 102 | 2.0 | 204 |
| 1 p. m. | 9965 | 36.0 | 3587 | 11.5 | 1145 | 47.5 | 4733 | 0.5 | 50 | 4.5 | 448 |
| 5 p. m. | 11490 | 40.0 | 4596 | 7.0 | 804 | 47.5 | 5457 | .. | .. | 5.5 | 631 |
| 3, 9 a. m. | 9875 | 28.0 | 2765 | 9.0 | 888 | 57.5 | 5678 | 1.0 | 99 | 4.5 | 434 |
| 4, 9 a. m. | 9520 | 29.0 | 2760 | 10.0 | 952 | 57.0 | 5426 | .. | .. | 4.0 | 380 |
| 5, 9 a. m. | 11090 | 32.5 | 3604 | 8.0 | 887 | 51.5 | 5711 | 3.5 | 388 | 4.5 | 499 |
| March 16, 9 a. m. | 10400 | 40.22 | 4191 | 16.88 | 1755 | 38.88 | 3836 | .. | .. | 6.0 | 624 |
| 1 p. m. | 12580 | 31.0 | 3899 | 11.0 | 1383 | 51.5 | 6478 | 0.5 | 63 | 6.0 | 754 |
| 5 p. m. | 12800 | 51.5 | 6592 | 10.0 | 1280 | 32.5 | 4160 | 0.5 | 64 | 5.5 | 704 |
| 17, 9 a. m. | 11280 | 33.0 | 3722 | 9.5 | 1071 | 53.5 | 6034 | .. | .. | 4.0 | 451 |
| 18, 9 a. m. | 9800 | 35.5 | 3479 | 10.5 | 1029 | 48.0 | 4704 | 1.0 | 98 | 5.0 | 490 |
| 19, 9 a. m. | 9560 | 32.5 | 3107 | 11.0 | 1051 | 50.0 | 4780 | 1.0 | 95 | 5.5 | 525 |
| March 21, 9 a. m. | 9580 | 30.5 | 2922 | 12.0 | 1149 | 52.5 | 5029 | .. | .. | 5.0 | 479 |
| 1 p. m. | 9880 | 38.0 | 3746 | 11.0 | 1084 | 47.0 | 4634 | .. | .. | 4.0 | 394 |
| 5 p. m. | 9060 | 28.5 | 2582 | 16.0 | 1449 | 53.5 | 4847 | 0.5 | 45 | 1.5 | 136 |
| 22, 9 a. m. | 6520 | 27.5 | 1793 | 11.25 | 733 | 55.75 | 3635 | .. | .. | 5.5 | 358 |
| 23, 9 a. m. | 9880 | 31.0 | 3062 | 4.5 | 444 | 60.5 | 5977 | 0.5 | 49 | 3.5 | 345 |
| 24, 9 a. m. | 11660 | 31.0 | 3614 | 4.5 | 524 | 60.0 | 6996 | .. | .. | 4.5 | 524 |
| April 6, 9 a. m. | 9920 | 39.0 | 3868 | 12.0 | 1190 | 45.5 | 4513 | .. | .. | 3.5 | 347 |
| (Inoc. 0.01 cc. serum intradermally.) | | | | | | | | | | | |
| 1 p. m. | 14460 | 46.5 | 6724 | 4.0 | 578 | 45.5 | 6579 | .. | .. | 4.0 | 578 |
| 5 p. m. | 15200 | 22.5 | 3420 | 6.5 | 988 | 68.5 | 10412 | .. | .. | 2.5 | 380 |
| 7, 9 a. m. | 11100 | 41.0 | 4551 | 13.5 | 1490 | 41.0 | 4551 | .. | .. | 4.5 | 499 |
| 8, 9 a. m. | 10300 | 27.0 | 2781 | 6.0 | 618 | 64.0 | 6592 | .. | .. | 3.0 | 309 |
| 9, 9 a. m. | 9700 | 41.5 | 4025 | 14.5 | 1406 | 39.5 | 3831 | .. | .. | 4.5 | 436 |
| Rabbit No. 24 | | | | | | | | | | | |
| March 2, 9 a. m. | 5905 | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| 1 p. m. | 8960 | 39.0 | 3260 | 13.5 | 1128 | 44.0 | 3678 | 0.5 | 41 | 3.0 | 250 |
| 5 p. m. | 7990 | 29.5 | 2357 | 9.0 | 719 | 56.5 | 4514 | 0.5 | 40 | 4.5 | 359 |
| 3, 9 a. m. | 5620 | 27.5 | 1545 | 15.0 | 843 | 56.5 | 3175 | .. | .. | 1.0 | 56 |
| 4, 9 a. m. | 6290 | 24.0 | 1509 | 11.0 | 692 | 61.0 | 3837 | .. | .. | 4.0 | 251 |
| 5, 9 a. m. | 5590 | 34.5 | 1928 | 7.5 | 419 | 55.5 | 3102 | .. | .. | 2.5 | 139 |
| March 16, 9 a. m. | 8880 | 20.5 | 1820 | 13.5 | 1198 | 56.0 | 4972 | 4.0 | 355 | 6.0 | 532 |
| 1 p. m. | 10060 | 35.2 | 3752 | 12.8 | 1364 | 44.0 | 4690 | 4.4 | 469 | 3.6 | 383 |
| 5 p. m. | 10220 | 37.0 | 3781 | 10.5 | 1073 | 42.0 | 4292 | 4.5 | 460 | 6.0 | 613 |
| 17, 9 a. m. | 7140 | 22.4 | 1599 | 16.0 | 1142 | 54.4 | 3884 | 5.6 | 400 | 1.6 | 114 |
| 18, 9 a. m. | 7500 | 33.5 | 2512 | 9.5 | 712 | 52.0 | 3900 | 3.0 | 225 | 2.0 | 150 |
| 19, 9 a. m. | 7160 | 32.0 | 2291 | 12.0 | 859 | 50.0 | 3580 | 3.0 | 214 | 3.0 | 214 |

*In all the tables the following abbreviations are used:
Wbc=white blood cells.
Pmn=polymorphonuclears.
Lm=large mononuclears.
Sm=small mononuclears.
Eos=eosinophiles.
Mst=mast cells.

Rabbit No. 24—Cont.

| | Total Leucocytes | Pmn % | Pmn Total | Lm % | Lm Total | Sm % | Sm Total | Eos % | Eos Total | Mst % | Mst Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| March 21, 9 a. m. | 6300 | 12.0 | 756 | 16.0 | 1008 | 65.0 | 4095 | 3.5 | 220 | 3.5 | 220 |
| (Inoc. 0.01 cc. serum intravenously.) | | | | | | | | | | | |
| 1 p. m. | 7500 | 35.5 | 2662 | 13.0 | 975 | 43.5 | 3262 | 1.0 | 75 | 7.0 | 525 |
| 5 p. m. | 8680 | 47.0 | 4079 | 8.0 | 694 | 38.5 | 3341 | 3.0 | 260 | 3.5 | 303 |
| 22, 9 a. m. | 5900 | 27.5 | 1622 | 12.0 | 708 | 56.0 | 3304 | 0.5 | 29 | 4.0 | 236 |
| 23, 9 a. m. | 5320 | 27.0 | 1436 | 5.5 | 292 | 59.5 | 3164 | 3.0 | 159 | 5.0 | 266 |
| 24, 9 a. m. | 5820 | 30.5 | 1775 | 9.5 | 553 | 51.5 | 2997 | 3.0 | 174 | 5.5 | 329 |
| April 6, 9 a. m. | 7100 | 27.0 | 1917 | 5.5 | 390 | 62.0 | 4402 | 2.0 | 142 | 3.5 | 248 |
| (Inoc. 0.01 cc. serum intradermally.) | | | | | | | | | | | |
| 1 p. m. | 7660 | 44.0 | 3370 | 8.0 | 612 | 42.5 | 3255 | 1.5 | 115 | 4.0 | 309 |
| 5 p. m. | 8940 | 30.5 | 2726 | 3.0 | 268 | 64.5 | 5766 | 1.0 | 89 | 1.0 | 89 |
| 7, 9 a. m. | 6760 | 41.0 | 2771 | 5.5 | 372 | 51.0 | 3447 | 2.0 | 135 | 0.5 | 34 |
| 8, 9 a. m. | 5680 | 34.0 | 1931 | 7.5 | 426 | 52.0 | 2953 | 4.0 | 227 | 2.5 | 142 |
| 9, 9 a. m. | 7000 | 45.5 | 3185 | 7.5 | 525 | 41.0 | 2870 | 2.0 | 140 | 4.0 | 280 |

Rabbit No. 92

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| March 2, 9 a. m. | 7580 | 57.0 | 4320 | 8.5 | 644 | 38.5 | 2539 | .. | .. | 1.0 | 76 |
| 1 p. m. | 9635 | 51.5 | 4962 | 9.0 | 867 | 38.0 | 3661 | 1.0 | 96 | 0.5 | 48 |
| 5 p. m. | 9670 | 63.5 | 6140 | 6.0 | 580 | 26.5 | 2562 | .. | .. | 4.0 | 387 |
| 3, 9 a. m. | 6840 | 61.0 | 4172 | 6.5 | 444 | 31.5 | 2154 | .. | .. | 1.0 | 68 |
| 4, 9 a. m. | 11160 | 51.5 | 5747 | 6.5 | 725 | 40.0 | 4464 | 1.0 | 111 | 1.0 | 111 |
| 5, 9 a. m. | 11310 | 76.0 | 8595 | 2.5 | 282 | 19.5 | 2205 | 0.5 | 56 | 1.5 | 169 |
| March 16, 9 a. m. | 6240 | 48.0 | 2995 | 9.0 | 561 | 37.0 | 2308 | 1.5 | 93 | 4.5 | 280 |
| 1 p. m. | 8040 | 34.5 | 2773 | 10.0 | 804 | 52.5 | 4221 | 0.5 | 40 | 2.5 | 201 |
| 5 p. m. | 6880 | 46.5 | 3199 | 9.0 | 619 | 42.0 | 2889 | .. | .. | 2.5 | 172 |
| 9 a. m. | 7600 | 35.0 | 2660 | 13.5 | 1026 | 50.5 | 3838 | .. | .. | 1.0 | 76 |
| 17, 9 a. m. | 9540 | 27.0 | 2576 | 9.5 | 906 | 60.5 | 5772 | 0.5 | 48 | 2.5 | 238 |
| 18, 9 a. m. | 6460 | 56.5 | 3650 | 4.5 | 290 | 36.5 | 2358 | 0.5 | 32 | 2.0 | 129 |
| March 21, 9 a. m. | 6740 | 37.0 | 2494 | 9.5 | 640 | 52.0 | 3505 | 0.5 | 33 | 1.0 | 67 |
| (Inoc. 1.0 cc. serum intravenously.) | | | | | | | | | | | |
| 1 p. m. | 6220 | 41.5 | 2581 | 6.5 | 404 | 48.0 | 2985 | 1.5 | 93 | 2.5 | 155 |
| 5 p. m. | 7440 | 56.0 | 4166 | 6.0 | 446 | 35.5 | 2641 | .. | .. | 2.5 | 186 |
| 22, 9 a. m. | 6780 | 42.0 | 2847 | 11.0 | 746 | 44.5 | 3017 | .. | .. | 2.5 | 169 |
| 23, 9 a. m. | 8260 | 39.0 | 3221 | 8.0 | 661 | 50.5 | 4171 | .. | .. | 2.5 | 206 |
| 24, 9 a. m. | 6260 | 30.0 | 1878 | 4.0 | 250 | 61.0 | 3819 | 0.5 | 31 | 4.5 | 282 |
| April 6, 9 a. m. | 6920 | 33.5 | 2318 | 4.5 | 311 | 62.0 | 4290 | .. | .. | .. | .. |
| (Inoc. 0.01 cc. serum intradermally.) | | | | | | | | | | | |
| 1 p. m. | 10060 | 53.0 | 5332 | 5.5 | 553 | 40.0 | 4024 | 0.5 | 50 | 1.0 | 100 |
| 5 p. m. | 7940 | 36.5 | 2898 | 2.5 | 198 | 60.0 | 4764 | 0.5 | 40 | 0.5 | 40 |
| 7, 9 a. m. | 6300 | 52.0 | 3276 | 10.5 | 661 | 34.5 | 2173 | 0.5 | 32 | 2.5 | 158 |
| 8, 9 a. m. | 8360 | 45.0 | 3762 | 8.0 | 669 | 45.5 | 3804 | 0.5 | 42 | 1.0 | 83 |
| 9, 9 a. m. | 5320 | 34.0 | 1809 | 5.0 | 266 | 58.5 | 3112 | 0.5 | 26 | 2.0 | 106 |

Rabbit No. 104

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| March 2, 9 a. m. | 7880 | 37.0 | 2915 | 8.5 | 670 | 51.5 | 4058 | .. | .. | 3.0 | 236 |
| 1 p. m. | 10650 | 40.5 | 4313 | 6.0 | 639 | 49.0 | 5218 | 1.0 | 106 | 3.5 | 372 |
| 5 p. m. | 10340 | 36.0 | 3722 | 11.5 | 1189 | 46.0 | 4756 | .. | .. | 6.5 | 672 |
| 3, 9 a. m. | 7460 | 56.0 | 4177 | 4.0 | 298 | 37.0 | 2760 | .. | .. | 3.0 | 223 |
| 4, 9 a. m. | 7160 | 33.5 | 2398 | 6.0 | 429 | 55.5 | 3974 | 0.5 | 36 | 4.5 | 322 |
| 5, 9 a. m. | 9000 | 30.5 | 2745 | 6.5 | 585 | 58.5 | 5265 | 1.5 | 135 | 3.0 | 270 |
| March 16, 9 a. m. | 8860 | 25.5 | 2259 | 8.0 | 709 | 57.5 | 5094 | 1.0 | 88 | 3.0 | 709 |
| 1 p. m. | 11160 | 42.0 | 4687 | 7.5 | 837 | 42.0 | 4687 | 5.5 | 613 | 3.0 | 334 |
| 5 p. m. | 13480 | 35.5 | 4785 | 12.5 | 1685 | 45.5 | 6133 | 2.5 | 337 | 4.0 | 539 |
| 17, 9 a. m. | 8760 | 33.0 | 2891 | 8.0 | 701 | 50.0 | 4380 | 4.5 | 394 | 4.5 | 394 |
| 18, 9 a. m. | 12180 | 37.0 | 4506 | 10.5 | 1279 | 41.5 | 5054 | 3.0 | 365 | 8.0 | 974 |
| 19, 9 a. m. | 10380 | 35.5 | 3685 | 12.0 | 1245 | 39.0 | 4048 | 3.5 | 882 | 5.0 | 519 |
| March 21, 9 a. m. | 5900 | 22.0 | 1298 | 14.5 | 855 | 55.5 | 3274 | 3.0 | 177 | 5.0 | 295 |
| (Inoc. 5.0 cc. serum intravenously.) | | | | | | | | | | | |
| 1 p. m. | 9480 | 66.0 | 6256 | 4.5 | 426 | 26.5 | 2512 | 1.5 | 142 | 1.5 | 142 |
| 5 p. m. | 7700 | 46.5 | 3580 | 7.5 | 577 | 37.5 | 2887 | 7.5 | 577 | 1.0 | 77 |
| 22, 9 a. m. | 9020 | 36.5 | 3292 | 11.5 | 1037 | 46.5 | 4194 | 3.0 | 270 | 2.5 | 225 |
| 23, 9 a. m. | 7940 | 45.0 | 3573 | 10.5 | 833 | 38.5 | 3057 | 1.0 | 79 | 5.0 | 394 |
| 24, 9 a. m. | 8520 | 41.5 | 3535 | 11.0 | 937 | 39.0 | 3322 | 0.5 | 42 | 8.0 | 684 |
| April 6, 9 a. m. | 6280 | 38.5 | 2417 | 10.0 | 628 | 44.5 | 2794 | 1.0 | 62 | 6.0 | 376 |
| (Inoc. 0.01 cc. serum intradermally.) | | | | | | | | | | | |
| 1 p. m. | 10960 | 70.0 | 7672 | 2.5 | 274 | 24.5 | 2685 | 0.5 | 54 | 2.5 | 274 |
| 5 p. m. | 10640 | 60.5 | 6437 | 2.0 | 212 | 34.5 | 3670 | 0.25 | 26 | 2.75 | 292 |
| 7, 9 a. m. | 9200 | 58.0 | 5336 | 8.0 | 736 | 32.5 | 2990 | 0.5 | 46 | 1.0 | 92 |
| 8, 9 a. m. | 8820 | 32.0 | 2822 | 8.0 | 705 | 56.0 | 4939 | .. | .. | 4.0 | 353 |
| 9, 9 a. m. | 8040 | 35.5 | 2854 | 4.0 | 321 | 56.5 | 4542 | 1.0 | 80 | 3.0 | 241 |

Chart V represents the total leucocyte counts for the four rabbits of the third series plotted out in such a way that one can compare the variations in the whole series at a glance. This chart shows that the variations in the leucocytes during the two periods of observation before inoculation, March 2-5 and March 16-19 were approximately as great as those following the intravenous injection of serum, March 21, and the cutaneous test, April 6, except in the case of rabbit No. 15, which was a control and did not receive the intravenous inoculation with the others on March 16. This is made clear by the following figures:

| | Two periods of observation before inoculation | | Following intravenous inoculation* | Following intradermal inoculation |
|---|---|---|---|---|
| | Mch. 2-5 | Mch. 16-19 | Mch. 21-24 | Apr. 6-9 |
| Rabbit No. 15: | | | | |
| Highest count.. | 11490 | 12800 | 11660 | 15200 |
| Lowest count.. | 9520 | 9560 | 6520 | 9700 |
| Variation ..... | 1970 | 3240 | 5140 | 5500 |
| Rabbit No. 24: | | | | |
| Highest count.. | 8360 | 10660 | 8680 | 8940 |
| Lowest count.. | 5620 | 7140 | 5320 | 5680 |
| Variation ..... | 2740 | 3520 | 3360 | 3260 |
| Rabbit No. 92: | | | | |
| Highest count.. | 11310 | 9540 | 8260 | 10060 |
| Lowest count.. | 6840 | 6240 | 6220 | 5320 |
| Variation ..... | 4470 | 3300 | 2040 | 4740 |
| Rabbit No. 104: | | | | |
| Highest count.. | 10650 | 12180 | 9480 | 10960 |
| Lowest count.. | 7160 | 8760 | 5900 | 6280 |
| Variation ..... | 3490 | 3420 | 3580 | 4680 |

This chart (V) shows in all cases an increase in the leucocytes at the count made either three hours or seven hours after feeding, with a fall by the next morning at 9 o'clock to somewhere near the original level. It seems quite reasonable to regard this increase as a digestive leucocytosis. The effect, if any, of the intravenous injection of serum, March 21, and the intradermal inoculation, April 6, is to depress to a slight extent the height of this digestive leucocytosis.

Charts VI, VII, VIII and IX represent the total number of leucocytes and absolute values of the polymorphonuclear, large, and small mononuclear leucocytes for rabbits Nos. 15, 24, 92 and 104, respectively. These charts show that in general an increase in the total leucocyte count is due to an increase in the two principal elements, the polymorphonuclear and small mononuclear leucocytes. In this series however, the polymorphonuclears as a rule undergo a greater increase than do the small mononuclears.

In order to determine if there is anything characteristic in the differential count following the intravenous injection of fairly large amounts of serum or the intradermal injection of minimal amounts we have analyzed the changes in the total counts and the number of polymorphonuclears and small mononuclears three hours and seven hours after injection. For purposes of comparison we have included the corresponding counts made before injection. The results are shown in the accompanying table, examination of which shows that an increase in the total count after the injection of serum may be due to an increase of either or both of the principal leucocytes and that this relationship does not differ from that entering into increases in the total count which occurred before the injection of any serum.

* Rabbit No. 15 was a control and did not receive an intravenous injection.

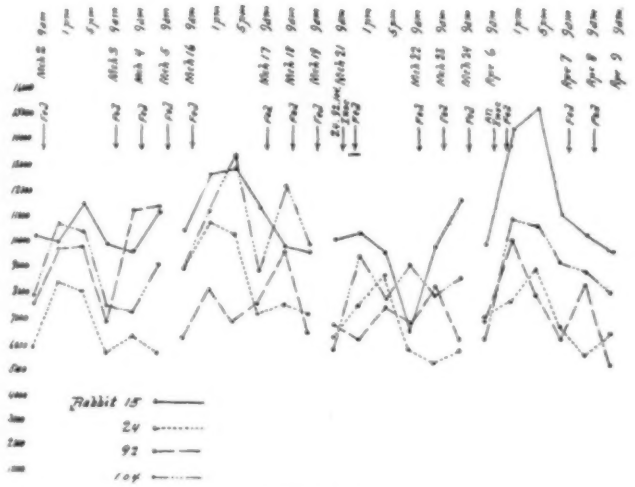


CHART V.

Rabbits Nos. 15, 24, 92 and 104 showing total leucocyte counts for entire period of observation. March 21 injected normal horse serum intravenously as follows: Rabbit No. 24, 0.1 cc.; Rabbit No. 92, 1 cc.; Rabbit No. 104, 5 cc. April 6 injected 0.01 cc. normal horse serum intradermally in all rabbits.

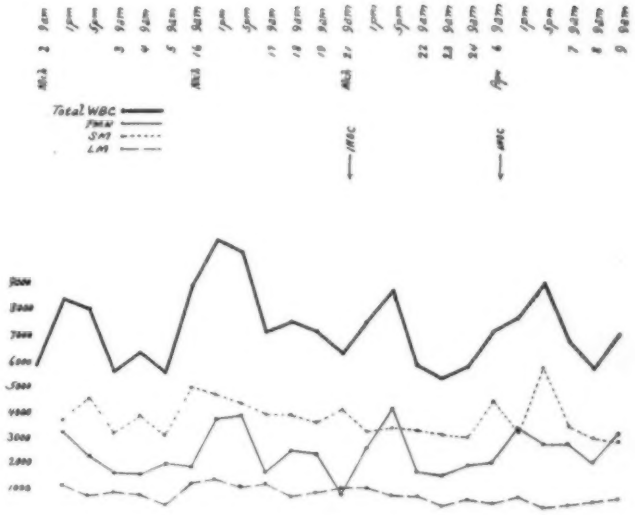


CHART VII.

Rabbit No. 24 showing total leucocyte counts and absolute values of polymorphonuclear, large and small mononuclear leucocytes for entire period of observation.

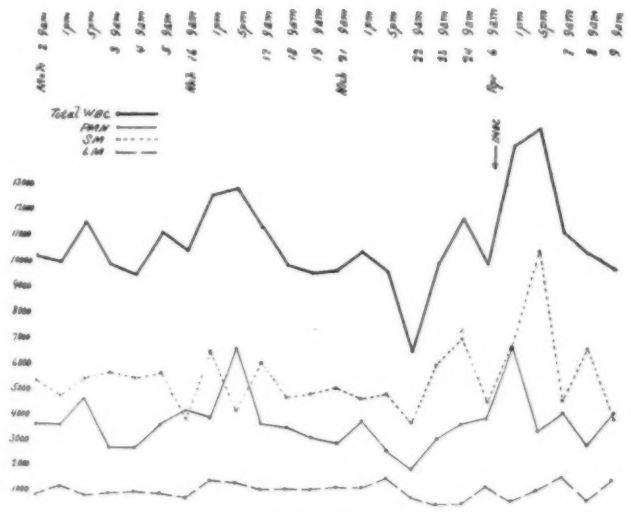


CHART VI.

Rabbit No. 15 showing total leucocyte counts and absolute values of polymorphonuclear, large and small mononuclear leucocytes for entire period of observation. April 6, 10 a. m., injected 0.01 cc. normal horse serum intradermally.

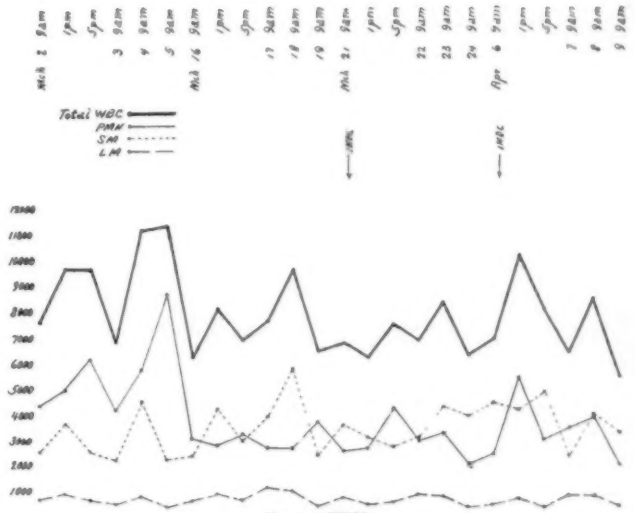


CHART VIII.

Rabbit No. 92 showing total leucocyte counts and absolute values of polymorphonuclear, large and small mononuclear leucocytes for entire period of observation.

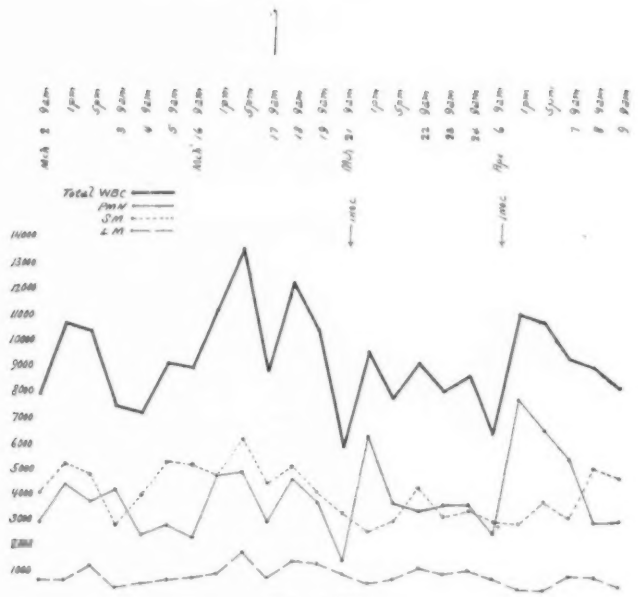


CHART IX.

Rabbit No. 104 showing total leucocyte counts and absolute values of polymorphonuclear, large and small mononuclear leucocytes for entire period of observation.

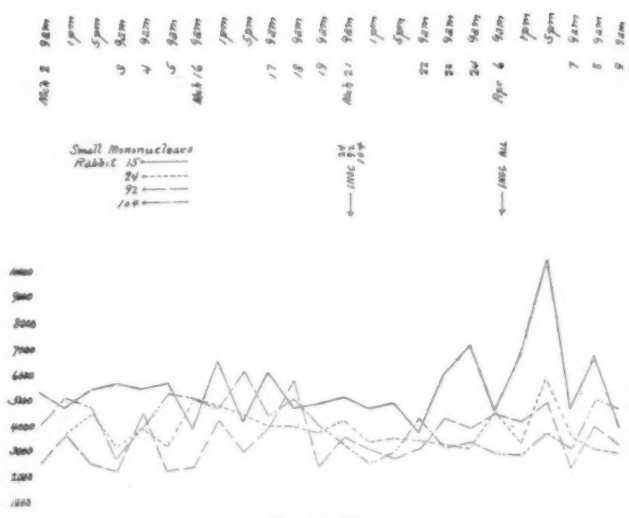


CHART XI.

Rabbits Nos. 15, 24, 92 and 104 showing absolute values of small mononuclear leucocytes.

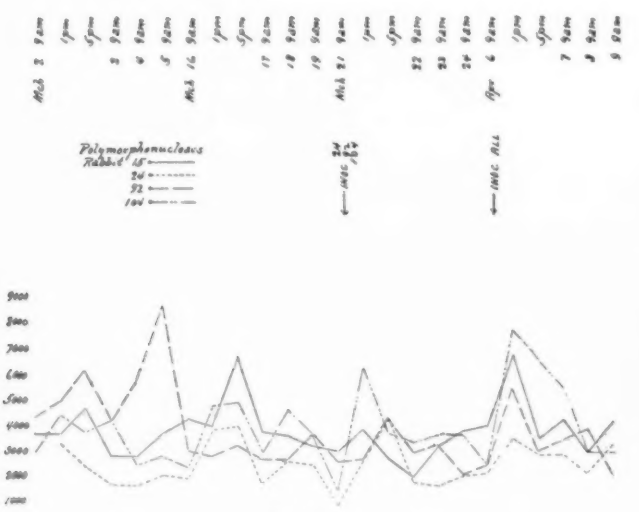


CHART X.

Rabbits Nos. 15, 24, 92 and 104 showing absolute values of polymorphonuclear leucocytes.

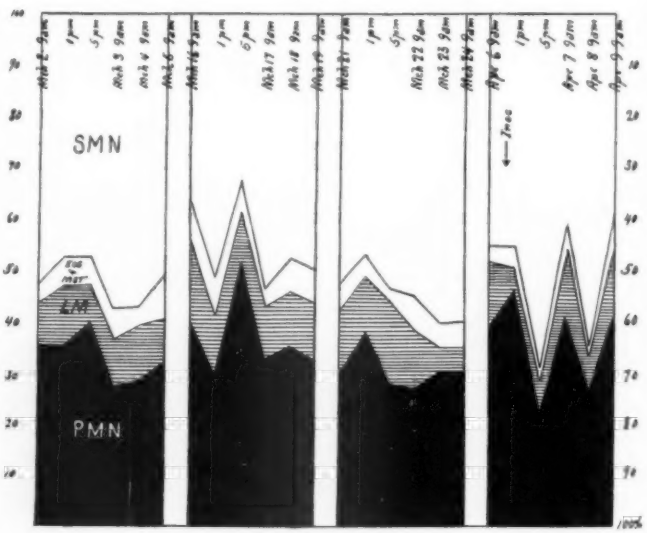


CHART XII.

Rabbit No. 15 showing percentage values of polymorphonuclear, large mononuclear, eosinophiles and mast cells combined, and small mononuclear leucocytes for entire period of observation.

| | Two periods of observation before inoculation | | | | Following intravenous inoculation* | | Following intradermal inoculation | |
|---|---|---|---|---|---|---|---|---|
| | March 2 | | March 16 | | March 21 | | April 6 | |
| | 1 p. m. | 5 p. m. | 1 p. m. | 5 p. m. | 1 p. m. | 5 p. m. | 1 p. m. | 5 p. m |
| Rabbit No. 15: | | | | | | | | |
| Total leucocytes. | — | + | + | + | + | — | + | + |
| Pmn ..... | ○ | + | — | + | + | — | + | — |
| Smn ..... | — | + | + | — | — | + | + | + |
| Rabbit No. 24: | | | | | | | | |
| Total leucocytes. | + | — | + | — | + | + | + | + |
| Pmn ..... | | — | + | + | + | + | + | — |
| Smn ..... | | + | — | — | — | ○ | — | + |
| Rabbit No. 92: | | | | | | | | |
| Total leucocytes. | + | ○ | + | — | — | + | + | — |
| Pmn ..... | + | + | — | + | + | + | + | — |
| Smn ..... | + | — | + | — | — | — | — | + |
| Rabbit No. 104: | | | | | | | | |
| Total leucocytes. | + | — | + | + | + | — | + | — |
| Pmn ..... | + | — | + | + | + | — | + | — |
| Smn ..... | + | — | — | + | — | + | — | + |

+ denotes an increase over the preceding count.
— denotes a decrease from the preceding count.
○ denotes no change from the preceding count.

For the purpose of better comparing the changes in the polymorphonuclear and small mononuclear counts, we have prepared Charts X and XI in which their curves have been superimposed. Chart X shows a definite rise in the polymorphonuclears of all rabbits three hours after the intradermal inoculation of 1/100 cc. serum; this rise, however, was participated in by rabbit No. 15, which had not been previously injected, as well as by the others which had been injected intravenously on March 21. Chart XI shows nothing remarkable except the sharp rise in the mononuclears of rabbit No. 15 on April 6 at 5 p. m., which we are unable to explain.

Charts XII, XIII, XIV, XV, show the percentage values of the differential counts on rabbits Nos. 15, 24, 92 and 104, respectively. The black areas at the bottom of the charts represent the percentage of polymorphonuclears; the shaded areas, the large mononuclears; the unshaded areas at the top of the charts, the small mononuclears; while the unshaded areas between the small and large mononuclears represent the eosinophiles and mast cells combined. These charts show that the relative numbers of large mononuclears, eosinophiles and mast cells do not undergo great variations, and that the relationship between the polymorphonuclears and small mononuclears is, in general, a reciprocal one; that is an increase in the percentage of polymorphonuclears is accompanied by a decrease in the percentage of small mononuclears and *vice versa*.

It is important to realize the different impression one gets from a consideration of the differential count expressed in percentages and in absolute values. To illustrate, suppose a total leucocyte count of 8000 cells, of which the polymorphonuclears are 60 per cent, small mononuclears 30 per cent, large mononuclears, eosinophiles, and mast cells combined 10 per cent. Now imagine the total count to increase to 10,000 cells due to an increase in the polymorphonuclears of 2000 cells. The percentage values now change to 68 per cent for the polymorphonuclears, 24 per cent small mononuclears, and 8 per cent mononuclears, eosinophiles and mast cells combined. How little idea one could get of the real change from a consideration of the percentages alone can be seen from this example;

| | | | |
|---|---|---|---|
| W. B. C. 8000 | Pmn ..... | 60% | = 4800 |
| | Sm ..... | 30 | = 2400 |
| | Lm, Eos and Mst..... | 10 | = 800 |
| W. B. C. 10000 | Pmn ..... | 68% | = 6800 |
| | Sm ..... | 24 | = 2400 |
| | Lm, Eos and Mst..... | 8 | = 800 |

In order to confirm the results of the preceding series we ran through a third series, consisting of rabbits Nos. 105, 106, 107 and 108. Of these No. 105 served as a control and received on sensitizing injection; the remaining three received intravenous injections of normal horse serum on June 16 at 10 a. m. as follows:

**Rabbit No. 106 1.0 cc. serum.**
**Rabbit No. 107 2.5 cc. serum.**
**Rabbit No. 108 5.0 cc. serum.**

The total and differential counts are given in the following tables:

THIRD SERIES.

| Rabbit No. 105 1910 | Total Leucocytes | Pmn % | Pmn Total | Lm % | Lm Total | Sm % | Sm Total | Eos % | Eos Total | Mst % | Mst Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| May 23, 9 a. m. | 9000 | 26.5 | 2623 | 3.5 | 347 | 60.5 | 5989 | 4.5 | 446 | 5.0 | 495 |
| 1 p. m. | 12140 | 28.5 | 3460 | 4.0 | 485 | 65.0 | 7891 | 1.5 | 182 | 1.0 | 121 |
| 5 p. m. | 12900 | 27.5 | 3547 | 3.5 | 451 | 63.5 | 8191 | 2.0 | 258 | 3.5 | 451 |
| 24, 9 a. m. | 9780 | 32.0 | 3129 | 5.0 | 489 | 59.0 | 5770 | 1.5 | 146 | 2.5 | 244 |
| 25, 9 a. m. | 9080 | 22.0 | 1997 | 6.0 | 545 | 66.0 | 5993 | 2.0 | 181 | 4.0 | 363 |
| 26, 9 a. m. | 8020 | 21.5 | 1724 | 5.5 | 441 | 69.0 | 5534 | 2.0 | 160 | 2.0 | 160 |
| June 1, 9 a. m. | 7540 | 21.0 | 1583 | 5.0 | 377 | 71.5 | 5391 | 1.0 | 75 | 1.5 | 113 |
| 1 p. m. | 10320 | 24.5 | 2528 | 11.5 | 1187 | 62.0 | 6398 | .. | .. | 2.0 | 206 |
| 5 p. m. | 11340 | 23.0 | 2608 | 5.5 | 624 | 70.0 | 7938 | .. | .. | 1.5 | 170 |
| 2, 9 a. m. | 10500 | 16.0 | 1680 | 7.0 | 735 | 71.5 | 7507 | .. | .. | 5.5 | 577 |
| 3, 9 a. m. | 9420 | 22.0 | 2072 | 2.5 | 235 | 71.5 | 6735 | 0.5 | 47 | 3.5 | 330 |
| 4, 9 a. m. | 10600 | 20.0 | 2120 | 5.0 | 530 | 72.5 | 7685 | .. | .. | 2.5 | 265 |
| June 16, 9 a. m. | 7720 | 36.0 | 2779 | 6.0 | 463 | 56.5 | 4362 | 0.5 | 38 | 1.0 | 77 |
| 1 p. m. | 6980 | 39.5 | 2757 | 5.5 | 384 | 53.0 | 3699 | .. | .. | 2.0 | 139 |
| 5 p. m. | 8000 | 33.0 | 2640 | 2.5 | 200 | 61.0 | 4880 | .. | .. | 3.5 | 280 |
| 17, 9 a. m. | 7940 | 26.5 | 2104 | 3.0 | 238 | 65.0 | 5161 | 0.5 | 40 | 5.0 | 397 |
| 18, 9 a. m. | 6800 | 21.5 | 1462 | 7.0 | 340 | 71.5 | 4862 | .. | .. | 2.0 | 136 |
| 19, 9 a. m. | 5300 | 26.5 | 1404 | 6.0 | 318 | 66.5 | 3524 | .. | .. | 1.0 | 53 |
| June 29, 9 a. m. | 8000 | 21.5 | 1861 | 4.0 | 346 | 69.0 | 5975 | 1.0 | 87 | 4.5 | 390 |
| (Inoc. 0.01 cc. serum intradermally.) | | | | | | | | | | | |
| 1 p. m. | 10250 | 27.6 | 2819 | 3.0 | 318 | 65.5 | 6838 | 1.0 | 104 | 3.5 | 365 |
| 5 p. m. | 11260 | 38.5 | 4335 | 4.5 | 507 | 55.0 | 6193 | .. | .. | 2.0 | 225 |
| 30, 9 a. m. | 9540 | 31.5 | 3005 | 5.0 | 477 | 59.5 | 5676 | .. | .. | 4.0 | 382 |
| July 1, 9 a. m. | 8560 | 25.5 | 2183 | 5.0 | 428 | 65.5 | 5607 | 1.0 | 85 | 3.0 | 257 |
| 2, 9 a. m. | 7440 | 33.5 | 2492 | 2.5 | 186 | 62.0 | 4613 | .. | .. | 2.0 | 149 |
| Rabbit No. 106 | | | | | | | | | | | |
| May 23, 9 a. m. | 4920 | 27.5 | 1353 | 7.5 | 369 | 64.0 | 3148 | .. | .. | 1.0 | 49 |
| 1 p. m. | 7140 | 29.5 | 2106 | 10.5 | 749 | 58.0 | 4141 | .. | .. | 2.0 | 143 |
| 5 p. m. | 7200 | 23.0 | 1656 | 9.0 | 648 | 67.0 | 4824 | .. | .. | 1.0 | 72 |
| 24, 9 a. m. | 6100 | 11.0 | 671 | 4.5 | 274 | 81.0 | 4941 | 2.5 | 152 | 1.0 | 61 |
| 25, 9 a. m. | 5580 | 29.5 | 1646 | 11.0 | 614 | 55.5 | 3097 | 0.5 | 28 | 3.5 | 195 |
| 26, 9 a. m. | 4660 | 21.0 | 978 | 12.0 | 559 | 62.5 | 2912 | 0.5 | 23 | 4.0 | 186 |
| June 1, 9 a. m. | 6060 | 22.5 | 1363 | 14.0 | 848 | 56.0 | 3393 | 0.5 | 30 | 7.0 | 424 |
| 1 p. m. | 8720 | 37.5 | 3270 | 6.5 | 567 | 51.0 | 4447 | .. | .. | 5.0 | 436 |
| 5 p. m. | 6560 | 36.0 | 2361 | 10.5 | 689 | 45.5 | 2985 | .. | .. | 8.0 | 525 |
| 2, 9 a. m. | 6720 | 31.5 | 2117 | 10.5 | 706 | 51.0 | 3427 | .. | .. | 7.0 | 470 |
| 3, 9 a. m. | 4360 | 32.0 | 1395 | 5.0 | 218 | 55.5 | 2420 | .. | .. | 7.5 | 327 |
| 4, 9 a. m. | 4360 | 47.5 | 2071 | 6.0 | 262 | 38.0 | 1657 | 1.0 | 43 | 7.5 | 327 |
| June 16, 9 a. m. | 4040 | 28.5 | 1151 | 7.0 | 283 | 55.5 | 2242 | 0.5 | 20 | 8.5 | 343 |
| (Inoc. 1.0 cc. serum intravenously.) | | | | | | | | | | | |
| 1 p. m. | 8300 | 47.5 | 3942 | 4.5 | 373 | 45.0 | 3735 | .. | .. | 3.0 | 249 |
| 5 p. m. | 5440 | 30.5 | 1964 | 7.0 | 451 | 59.5 | 3832 | .. | .. | 3.0 | 193 |
| 17, 9 a. m. | 3800 | 33.5 | 1273 | 6.5 | 247 | 54.0 | 2052 | 0.5 | 19 | 5.5 | 209 |
| 18, 9 a. m. | 4760 | 45.5 | 2166 | 10.5 | 500 | 39.0 | 1836 | .. | .. | 5.0 | 238 |
| 19, 9 a. m. | 4920 | 39.0 | 1919 | 7.0 | 344 | 48.0 | 2361 | .. | .. | 6.0 | 295 |
| June 29, 9 a. m. | 5580 | 47.5 | 2650 | 7.0 | 390 | 40.0 | 2232 | 1.5 | 83 | 4.0 | 223 |
| (Inoc. 0.01 cc. serum intradermally.) | | | | | | | | | | | |
| 1 p. m. | 6460 | 37.0 | 2389 | 6.5 | 420 | 55.5 | 3585 | .. | .. | 1.0 | 65 |
| 5 p. m. | 6250 | 33.0 | 2062 | 10.5 | 656 | 46.0 | 2875 | 1.0 | 63 | 9.5 | 594 |
| 30, 9 a. m. | 6780 | 44.5 | 3017 | 10.5 | 712 | 35.5 | 2407 | 1.0 | 68 | 8.5 | 576 |
| July 1, 9 a. m. | 7380 | 37.5 | 2767 | 8.5 | 627 | 47.0 | 3469 | 1.5 | 111 | 5.5 | 406 |
| 2, 9 a. m. | 4720 | 40.0 | 1888 | 6.5 | 307 | 49.0 | 2313 | 0.5 | 23 | 4.0 | 189 |
| Rabbit No. 107 | | | | | | | | | | | |
| May 23, 9 a. m. | 4600 | 38.5 | 1771 | 13.0 | 598 | 44.0 | 2024 | .. | .. | 4.5 | 207 |
| 1 p. m. | 7480 | 29.5 | 2206 | 12.5 | 935 | 52.0 | 3889 | .. | .. | 6.0 | 449 |
| 5 p. m. | 9440 | 27.5 | 2596 | 10.5 | 991 | 56.5 | 5333 | 0.5 | 47 | 5.0 | 472 |
| 24, 9 a. m. | 4440 | 24.5 | 1088 | 13.0 | 577 | 60.0 | 2664 | .. | .. | 2.5 | 111 |
| 25, 9 a. m. | 5580 | 29.0 | 1618 | 14.0 | 781 | 55.0 | 3069 | .. | .. | 2.0 | 112 |
| 26, 9 a. m. | 5840 | 32.5 | 1898 | 11.0 | 642 | 50.0 | 2920 | .. | .. | 6.5 | 380 |

* Rabbit No. 15 served as a control and did not receive intravenous inoculation.

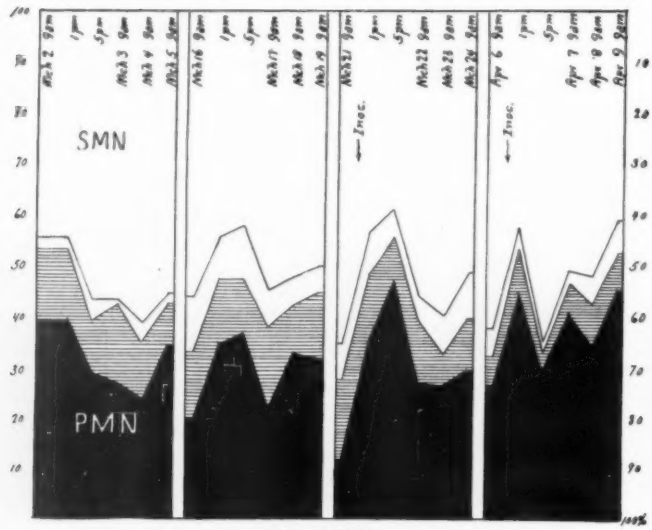


CHART XIII.

Rabbit No. 24 showing percentage values of polymorphonuclear, large mononuclear, eosinophiles and mast cells combined, and small mononuclear leucocytes for entire period of observation.

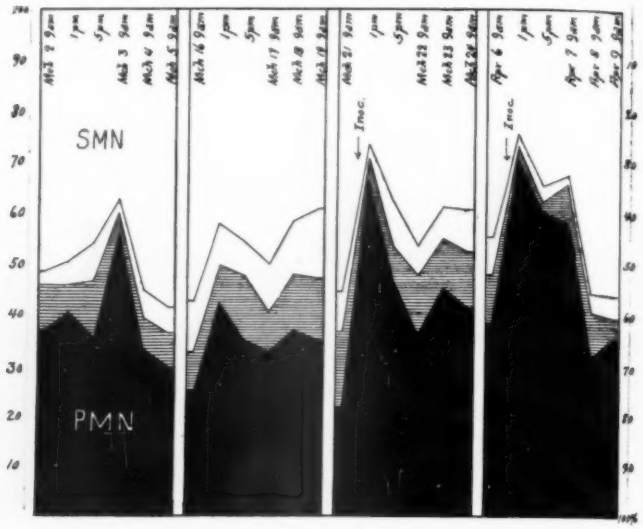


CHART XV.

Rabbit No. 104 showing percentage values of polymorphonuclear, large mononuclear, eosinophiles and mast cells combined, and small mononuclear leucocytes for entire period of observation.

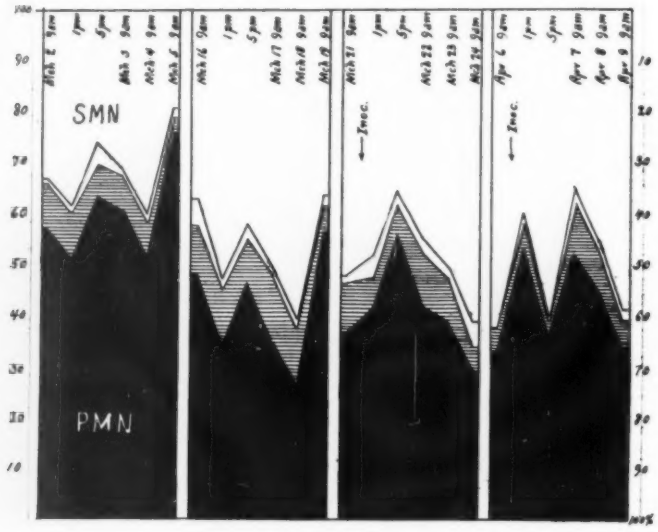


CHART XIV.

Rabbit No. 92 showing percentage values of polymorphonuclear, large mononuclear, eosinophiles and mast cells combined, and small mononuclear leucocytes for entire period of observation.

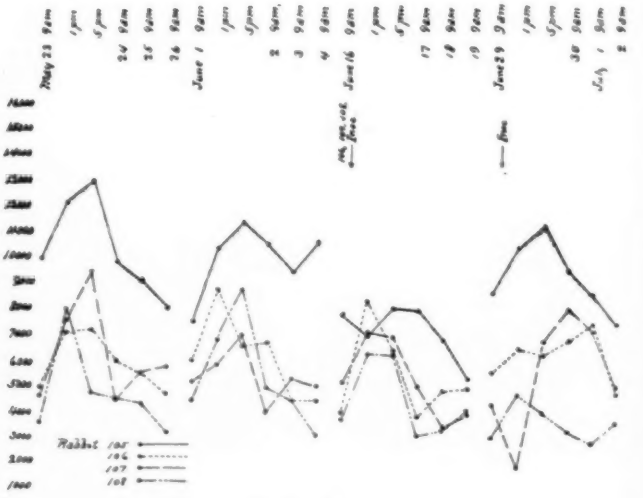


CHART XVI.

Rabbits Nos. 105, 106, 107 and 108 showing total leucocyte counts for entire period of observation. June 16 injected normal horse serum intravenously as follows: Rabbit No. 106, 1 cc.; Rabbit No. 107, 2.5 cc.; Rabbit No. 108, 5 cc. June 29 injected 0.01 cc. normal horse serum intradermally in all rabbits.

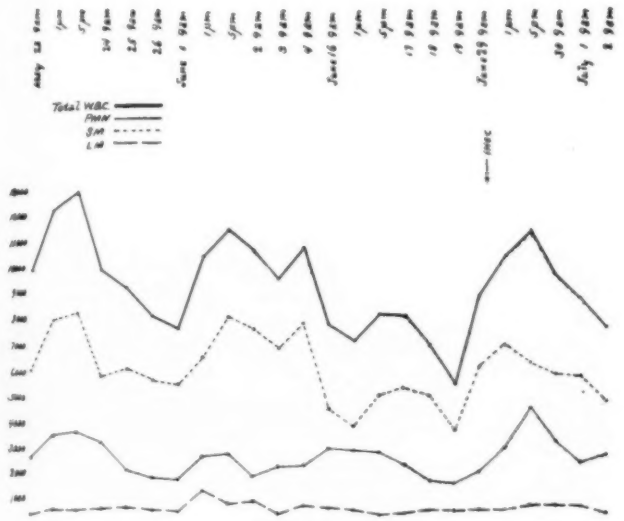

CHART XVII.

Rabbit No. 105 showing total leucocyte counts and absolute values of polymorphonuclear, large and small mononuclear leucocytes for entire period of observation.

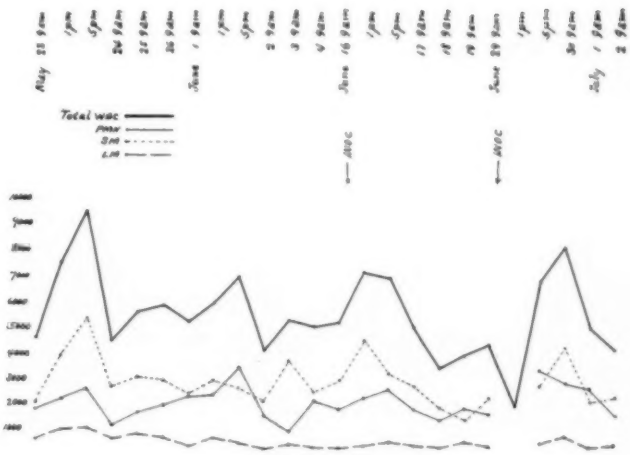

CHART XIX.

Rabbit No. 107 showing total leucocyte counts and absolute values of polymorphonuclear, large and small mononuclear leucocytes for entire period of observation.

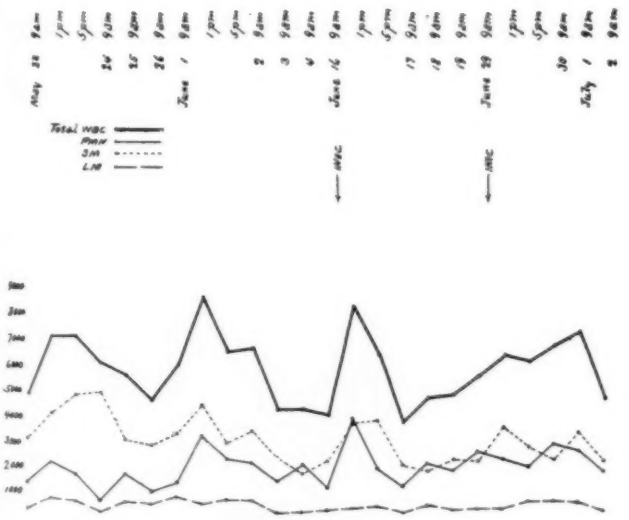

CHART XVIII.

Rabbit No. 106 showing total leucocyte counts and absolute values of polymorphonuclear, large and small mononuclear leucocytes for entire period of observation.

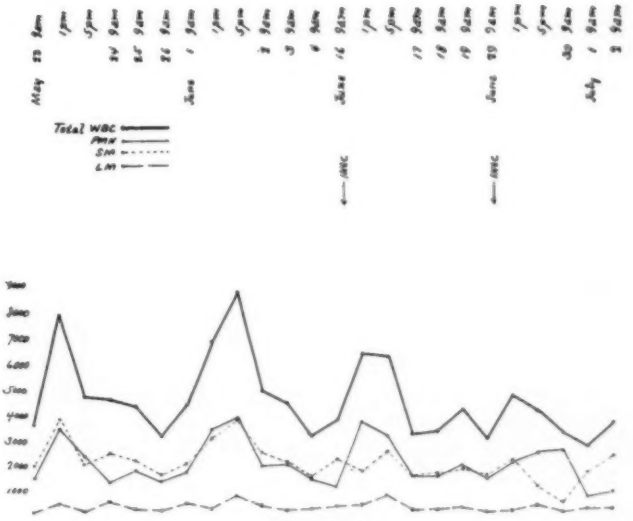

CHART XX.

Rabbit No. 108 showing total leucocyte counts and absolute values of polymorphonuclear, large and small mononuclear leucocytes for entire period of observation.

Rabbit No. 107—Cont.

| | | Total Leucocytes | Pmn % | Pmn Total | Lm % | Lm Total | Sm % | Sm Total | Eos % | Eos Total | Mst % | Mst Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| June | 1, 9 a. m. | 5180 | 43.5 | 2253 | 6.0 | 311 | 45.5 | 2357 | 1.5 | 78 | 3.5 | 181 |
| | 1 p. m. | 5940 | 38.5 | 2287 | 10.5 | 624 | 49.0 | 2910 | .. | .. | 2.0 | 119 |
| | 5 p. m. | 6960 | 48.5 | 3375 | 5.5 | 383 | 37.5 | 2610 | 0.5 | 35 | 8.0 | 557 |
| | 2, 9 a. m. | 4040 | 35.5 | 1434 | 4.5 | 182 | 50.5 | 2040 | 1.0 | 40 | 8.5 | 343 |
| | 3, 9 a. m. | 5260 | 16.0 | 842 | 8.0 | 421 | 70.0 | 3682 | 0.5 | 26 | 5.5 | 289 |
| | 4, 9 a. m. | 5000 | 41.0 | 2050 | 4.5 | 225 | 49.0 | 2450 | .. | .. | 5.5 | 275 |
| June | 16, 9 a. m. | 5200 | 33.0 | 1716 | 5.0 | 260 | 55.5 | 2886 | 0.5 | 26 | 6.0 | 312 |
| | (Inoc. 2.5 cc. serum intravenously.) | | | | | | | | | | | |
| | 1 p. m. | 7140 | 30.0 | 2142 | 4.0 | 286 | 62.5 | 4462 | 0.5 | 36 | 3.0 | 214 |
| | 5 p. m. | 6860 | 37.0 | 2538 | 6.5 | 446 | 46.0 | 3156 | .. | .. | 10.5 | 720 |
| | 17, 9 a. m. | 5020 | 34.0 | 1707 | 7.0 | 351 | 53.0 | 2661 | .. | .. | 6.0 | 301 |
| | 18, 9 a. m. | 3420 | 37.5 | 1283 | 7.0 | 239 | 51.5 | 1761 | .. | .. | 4.0 | 137 |
| | 19, 9 a. m. | 3860 | 45.5 | 1756 | 11.0 | 425 | 34.0 | 1312 | .. | .. | 9.5 | 367 |
| June | 29, 9 a. m. | 4300 | 35.0 | 1505 | 7.5 | 323 | 51.0 | 2193 | .. | .. | 6.5 | 279 |
| | (Inoc. 0.01 cc. serum intradermally.) | | | | | | | | | | | |
| | 1 p. m. | 1800 | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| | 5 p. m. | 6780 | 49.0 | 3322 | 6.0 | 407 | 39.0 | 2644 | .. | .. | 6.0 | 407 |
| | 30, 9 a. m. | 8070 | 35.0 | 2824 | 8.0 | 646 | 52.0 | 4196 | 0.5 | 40 | 4.5 | 363 |
| July | 1, 9 a. m. | 4960 | 52.0 | 2579 | 5.0 | 248 | 40.0 | 1984 | .. | .. | 3.0 | 149 |
| | 2, 9 a. m. | 4140 | 35.5 | 4170 | 7.0 | 290 | 53.0 | 2194 | 1.0 | 41 | 3.5 | 145 |
| Rabbit No. 108 | | | | | | | | | | | | |
| May | 23, 9 a. m. | 3600 | 40.0 | 1440 | 4.0 | 144 | 54.0 | 1944 | 1.0 | 36 | 1.0 | 36 |
| | 1 p. m. | 7920 | 43.5 | 3445 | 5.5 | 436 | 48.0 | 3801 | 0.5 | 40 | 2.5 | 198 |
| | 5 p. m. | 4700 | 51.5 | 2420 | 4.0 | 188 | 43.0 | 2021 | .. | .. | 1.5 | 71 |
| | 24, 9 a. m. | 4540 | 28.5 | 1294 | 12.5 | 567 | 54.5 | 2484 | .. | .. | 4.5 | 204 |
| | 25, 9 a. m. | 4280 | 40.5 | 1733 | 6.0 | 257 | 51.5 | 2204 | 0.5 | 21 | 1.5 | 64 |
| | 26, 9 a. m. | 3160 | 41.0 | 1296 | 6.0 | 190 | 48.5 | 1532 | 2.0 | 63 | 2.5 | 79 |
| June | 1, 9 a. m. | 4420 | 39.2 | 1654 | 10.8 | 456 | 46.8 | 1975 | 0.8 | 33 | 2.4 | 101 |
| | 1 p. m. | 6840 | 49.5 | 3386 | 4.0 | 273 | 44.5 | 3044 | 1.0 | 68 | 1.0 | 68 |
| | 5 p. m. | 8700 | 44.5 | 3871 | 8.5 | 739 | 43.5 | 3784 | 1.5 | 131 | 2.0 | 174 |
| | 2, 9 a. m. | 4920 | 39.0 | 1919 | 8.5 | 418 | 50.5 | 2484 | 0.5 | 25 | 1.5 | 74 |
| | 3, 9 a. m. | 4380 | 44.5 | 1949 | 4.0 | 175 | 47.0 | 2059 | .. | .. | 4.5 | 197 |
| | 4, 9 a. m. | 3120 | 43.0 | 1341 | 8.0 | 250 | 46.0 | 1435 | .. | .. | 3.0 | 94 |
| June | 16, 9 a. m. | 3740 | 30.0 | 1122 | 8.5 | 318 | 59.0 | 2206 | 0.5 | 19 | 2.0 | 75 |
| | (Inoc. 5.0 cc. serum intravenously.) | | | | | | | | | | | |
| | 1 p. m. | 6340 | 58.0 | 3677 | 6.5 | 412 | 26.5 | 1680 | 0.5 | 32 | 8.5 | 539 |
| | 5 p. m. | 6260 | 49.5 | 3099 | 12.0 | 751 | 36.0 | 2254 | .. | .. | 2.5 | 156 |
| | 17, 9 a. m. | 3140 | 45.5 | 1429 | 6.5 | 204 | 45.5 | 1429 | 0.5 | 16 | 2.0 | 62 |
| | 18, 9 a. m. | 3320 | 43.5 | 1444 | 5.5 | 183 | 47.5 | 1577 | .. | .. | 3.5 | 116 |
| | 19, 9 a. m. | 4080 | 46.5 | 1897 | 6.5 | 265 | 43.0 | 1754 | 1.0 | 41 | 3.0 | 122 |
| June | 29, 9 a. m. | 3020 | 45.0 | 1359 | 2.5 | 75 | 50.0 | 1510 | 0.5 | 15 | 2.0 | 60 |
| | (Inoc. 0.01 cc. serum intradermally.) | | | | | | | | | | | |
| | 1 p. m. | 4660 | 43.0 | 2004 | 4.5 | 210 | 46.5 | 2167 | 3.5 | 163 | 2.5 | 116 |
| | 5 p. m. | 4040 | 60.5 | 2444 | 10.0 | 404 | 27.5 | 1111 | 0.5 | 20 | 1.5 | 60 |
| | 30, 9 a. m. | 3260 | 78.0 | 2543 | 3.0 | 98 | 13.5 | 440 | 0.5 | 16 | 5.0 | 163 |
| July | 1, 9 a. m. | 2760 | 25.5 | 704 | 9.5 | 262 | 61.0 | 1683 | 0.5 | 14 | 3.5 | 96 |
| | 2, 9 a. m. | 3620 | 25.5 | 923 | 6.5 | 235 | 64.0 | 2317 | 1.0 | 36 | 3.0 | 108 |

Chart XVI shows the total leucocyte counts for the entire series plotted out so that a general idea of the variations which have taken place can be gotten at a glance.

One sees that the leucocytes underwent as great changes in number during the two series of observations before inoculation as they did after inoculation. On May 23, June 1, 16 and 29, days on which counts were made at 9 a. m., 1 p. m. and 5 p. m., it is to be observed that the 1 p. m. count, or the 5 p. m. count, or both, are higher than the 9 a. m. count. This we ascribe to a digestive leucocytosis as in the preceding series, the animals being fed at 10 a. m. each day. We are unable to see that the intravenous injection of normal horse serum exerted any influence on the total number of leucocytes. It is possible that the depression of the digestive leucocytosis which is observable on June 29 may have been due to the intradermal inoculation.

Charts XVII, XVIII, XIX and XX show the total leucocytes and absolute values of the differential counts plotted out for rabbits Nos. 105, 106, 107 and 108, respectively. They illustrate very well the observation made in connection with the preceding series; namely, that both the polymorphonuclear and small mononuclear leucocytes participate in an increase in the total number of leucocytes, but in this series the small mononuclears, as a rule, participate to a greater extent than do the polymorphonuclears.* It is possible that the exception to this rule observable in Charts XVIII and XX, June 16, 1 p. m., where we see a relatively greater increase in the polymorphonuclear cells may have been due to the intravenous injection of normal horse serum, but inasmuch as similar exceptions are to be seen elsewhere in the charts where no horse serum was inoculated, it would hardly be safe to draw conclusions from these two instances.

Curves showing the total number of polymorphonuclear leucocytes for the entire series were superimposed for purpose of comparison and the same was done for the small mononuclears, but as the injection of serum seems to have been entirely without influence on either of these elements the charts are omitted.

* These results are in entire agreement with those obtained in a fourth series of counts which are not included in this report on account of lack of space.

## Summary.

We have thought it worth while to publish these studies in full, giving the detailed counts for each series, since they represent a large number of carefully made observations and the data may be useful to others who are attempting to draw conclusions from variations of the leucocyte counts in rabbits.

We feel justified in saying that the normal variation in the total leucocyte count taken at the same hour each day of rabbits kept as nearly as possible under constant conditions is very great, at times reaching nearly 100 per cent.

We have observed quite regularly a diurnal cycle in which we are inclined to ascribe the increase in the number of leucocytes to the influence of digestion.

The relative and absolute values of the differential count vary under normal conditions from day to day and at different hours of the same day.

We observed that an increase in the total leucocyte count was participated in, as a rule, by both the polymorphonuclear and small mononuclear leucocytes, but usually by the latter to a greater extent than by the former.

The relative values (percentage values) of the polymorphonuclear and small mononuclear leucocytes bear a reciprocal relation to each other; that is, an increase in the percentage of one is accompanied by a decrease in the percentage of the other.

We were unable to observe that the intravenous injection of normal horse serum in doses varying from 0.1 cc. to 5 cc. or the intradermal injection of 0.01 cc. normal horse serum in either sensitized or non-sensitized rabbits had any definite or constant influence on the total or differential leucocyte counts, three hours or longer after injection.

We have a few observations, not included in this paper, where counts were made at short intervals (5-10 minutes) after the intravenous injection of large amounts (10 cc.) normal horse serum into rabbits. These indicate, as far as they go, that shortly after injection the total count undergoes a sharp decline with a marked relative increase of polymorphonuclear and decrease of small mononuclear leucocytes. These observations, however, were few in number and we hesitate to draw conclusions from them.

# THE USE OF ANTIFORMIN IN THE EXAMINATION OF SPUTUM FOR THE TUBERCLE BACILLUS.

By W. W. BOARDMAN, M. D.

*(From the Research Laboratory, The Phipps Tuberculosis Dispensary, The Johns Hopkins Hospital.)*

An absolute diagnosis of pulmonary tuberculosis can be based upon one, and only one finding—the demonstration of tubercle bacilli in the sputum. Yet in a review of the reports of various tuberculosis dispensaries and sanatoria we find that in the cases clinically diagnosed as definite pulmonary tuberculosis, the sputum is negative for tubercle bacilli in from 8 to 46 per cent. Are we to conclude then, that in from 8 to 46 per cent of our clinically positive cases, there are no tubercle bacilli in the sputum, and that these cases therefore are negligible factors in the spread of the disease; or that in a large proportion of these cases we fail to demonstrate tubercle bacilli, not because of their absence, but as a result of our imperfect methods of sputum examination, or our too careless use of these methods?

As to the first possibility; clinically positive cases without tubercle bacilli in the sputum do exist among the arrested cases and possibly in the very early stages of the disease, but there is no doubt that the number of cases so classed at the present time is too large.

The second possibility seems a more probable explanation. We fail to find tubercle bacilli in the sputum of many clinically positive cases, since (a) the specimen examined may contain no tubercle bacilli, being merely mucus from the upper air passages; or (b) if the tubercle bacilli are present in but small numbers, they may be overlooked, even in the most careful examination by the ordinary methods. This point is well illustrated by the following report by Goerres. In examining four smears from each of 296 specimens—96 were demonstrated to contain tubercle bacilli by the first smear, 24 more by the second, 9 more by the third, and 5 more by the fourth, and yet of the remaining 162 apparently negative sputa, 28 were demonstrated to contain tubercle bacilli by the antiformin method. Finally (c) as to the careless use of our present methods, it is evident, that if some cases escape detection even with repeated smear examinations, many may escape detection when but a single smear is made.

What then is the solution of the problem? It is evident that we must first obtain a specimen from the tuberculous lesion; again we must examine several smears from the same specimen; and lastly we must examine repeated specimens. The observance of these rules calls for the expenditure of much time and energy, and in return, although the results are fairly accurate, they still are far from perfect.

A more nearly absolute method, first proposed by Biedert[1] and slightly modified by subsequent investigators (Goerres[2]) has long been recognized as a valuable procedure, but the tedious technique has prevented its use except in a comparatively few doubtful cases. Briefly, the method is as follows:

A large quantity of sputum is well diluted with distilled water and 0.2 per cent sodium hydroxide is added, the material heated, stirring meanwhile, until a homogeneous solution results. It is then neutralized with acetic acid, and double the amount of 96 per cent alcohol added. It is then allowed to settle, the supernatant fluid is poured off and the sediment examined in the usual way. This method facilitates the finding of the bacilli by concentrating them in the sediment.

Following Biedert's first publication various agents were recommended for dissolving the mucus, such as hydrogen peroxide, carbolic acid, and pancreatic ferment, but none of these give so satisfactory results as the weak solution of sodium hydroxide with heat.

Thus matters stood until in the latter part of 1908, when Uhlenhuth[3] reported the results of his extensive researches with antiformin, a preparation much used by brewers in the disinfection of their fermentation vats and pipes.

Antiformin is a clear yellowish liquid of high specific gravity, possessing a faint chlorine odor and a decided soapy feel. According to Uhlenhuth it is composed of sodium hydroxide 7.5 per cent with sodium hypochlorite in such amount that 100 grams of antiformin liberates five and three-tenths grams of chlorine gas. After months of standing in the laboratory there is no appreciable deterioration.

As a result of his researches Uhlenhuth recommends antiformin as a disinfectant of the first rank, surpassing in many respects carbolic acid and bichloride of mercury. Its efficiency depends upon an intensive oxidation process, this being so marked that practically all organic matter except hair, wax, fat and cellulose, is rapidly brought into solution—the rate and completeness depending upon the strength of the solution used and the temperature at which the reaction occurs.

Upon adding a solution of antiformin to sputum, there is a marked liberation of gas. The sputum rises to the top of the mixture and rapidly dissolves, the result being a practically homogeneous solution, varying in color from yellowish-brown to a pale yellow, with but a small amount of flocculent sediment. The consistency of the resulting liquid varies directly with the consistency of the original specimen. The sediment varies in color from white to a dirty gray and consists of a finely granular detritus, fat needles, dust particles, cellulose fiber and any acid-fast organisms which may have been present in the original specimen.

A similar reaction occurs upon adding antiformin to finely divided animal tissues, pus, feces, suspensions of bacteria, etc. Uhlenhuth concludes that with the exception of one group of bacteria, all bacteria, protozoa, spirochætæ, and trypanosoma are dissolved in 2 to 5 per cent antiformin solution in from two and one-half to five minutes, the majority of them undergoing almost instantaneous solution. The organisms of the acid-fast group are the only ones resisting the dissolving

action of the antiformin solutions and this is explained by the fact that they are enveloped in a waxy capsule. Further work by Uhlenhuth and others revealed the important fact that tubercle bacilli, as shown by animal experimentation, are rapidly killed by antiformin only in 50 per cent or stronger solutions; that they retain their virulence for 12 days in 8 per cent solutions and for 4 days in 20 per cent solutions; finally that their staining properties are practically unaffected by exposure to 50 per cent antiformin for weeks.

We therefore have in antiformin an agent capable of rapidly and completely reducing sputum to a practically homogeneous solution without altering the staining qualities or viability of the contained tubercle bacilli. Several methods have been proposed for the recovery of the bacilli from these solutions, thereby making the procedure of practical value.

Uhlenhuth recommends simple sedimentation, his method for sputum examination being briefly as follows:

1. 15-30 cc. of sputum in a conical settling glass.
2. Add from three to five times the volume of water.
3. Add sufficient antiformin to make a 15 per cent solution.
4. Stir thoroughly and allow to digest and settle completely.
5. Pour off supernatant fluid.
6. Wash sediment with sterile water and allow sediment to reaccumulate.
7. Pour off supernatant fluid; smear sediment on slide and examine in usual manner.

The washing was found necessary since the presence of any considerable quantity of antiformin prevented firm fixing of the sediment to the slide.

Hüne[4] uses both the gravity sedimentation and the centrifuge. He proposes two distinct methods, as follows:*

I.

1. To a large quantity of sputum add antiformin to a 25 per cent solution.
2. After the production of a homogeneous solution add 96 per cent alcohol, thereby reducing the specific gravity and hastening sedimentation.
3. Pour off supernatant fluid.
4. Wash sediment in centrifuge.
5. Smear and examine.

II.

1. Take equal parts of sputum and sterile water.
2. Add antiformin to 2 per cent solution.
3. Allow to digest and sediment to accumulate.
4. Pour off supernatant fluid and smear directly.

In II washing is not necessary owing to the low percentage of antiformin; on the other hand, this process requires about three days for completion.

Meyer[5] also proposes two methods practically identical with the preceding:

I.

1. To the sputum add antiformin to a 20 per cent solution.
2. Transfer the entire resulting solution to centrifuge tubes and centrifugalize violently.
3. Wash sediment twice in centrifuge.
4. Smear and examine.

II.

1. To the sputum add antiformin to 3 per cent solution—examine sediment without washing.

Seeman[6] uses either the centrifuge method or gravity sedimentation. He also does away with the necessity of washing the sediment by using either diluted egg albumin or better, some of the original sputum to aid in fixing the smear.

METHOD.

1. To the sputum add antiformin to a 15-20 per cent solution.
2. Add 96 per cent alcohol after digestion is complete.
3. Smear sediment directly, using either egg albumin or some of the original sputum; or
2. Centrifuge the material.
3. Smear as in 3.

In using some of the original sputum to aid in fixing the smear, Seeman not only does away with the necessity of washing the sediment, but at the same time gains some insight into the general composition of the sputum, as regards the number and character of the other organisms, the type of cell predominating, and the presence or absence of elastic fibers; and also renders the search for the tubercle bacillus much easier by having the blue field upon which to focus.

Thilenius[7] uses the centrifuge after first having added alcohol to the sputum-antiformin mixture. He also uses antiformin in from 10-50 per cent solution.

Haserodt[8] recommends the use of ligroin, a light hydrocarbon oil, for obtaining the tubercle bacilli. His method is as follows:

1. To the sputum add antiformin to make 5 per cent solution.
2. Add a few cubic centimeters of ligroin and shake till an emulsion is produced.
3. Allow to stand until a sharp line of separation occurs between the ligroin and the sputum-antiformin mixture.
4. With platinum needle remove several loopsful of material from just below the ligroin; spread this on a slide and examine in the usual manner.

The tubercle bacilli and other undissolved particles in the mixture are carried upward by the little droplets of ligroin and deposited just below the line of separation between these two solutions. Haserodt claims that the smears, by this method, show no tendency to wash off the slides, but this statement is not upheld by other investigators. The method has no advantage over the previous ones, and has failed to give as good results.

Bernhardt's[9] procedure is exactly the same as Haserodt's, except he uses 5 cc. of sputum and adds 20 cc. of a 20 per cent solution of antiformin.

*Hüne substituted a mixture of sodium hypochloride 11.1% and potassium hydroxide 5.6% for antiformin with satisfactory results.

Goerres (*loc. cit.*), after carefully reviewing the work of other investigators, finally adopted the following method:

1. Equal parts of sputum and water.
2. Add antiformin to a 25 per cent solution, shake violently.
3. After 4-6 hours gently turn vessel to dislodge any particles which may be adhering to the side of the vessel.
4. Allow to settle for several hours.
5. Pour off supernatant fluid.
6. Fix sediment to slide with egg albumin or some of the original sputum.

The conclusions, finally reached from a review of the several articles dealing with the use of antiformin in sputum examinations, may be briefly summarized as follows:

1. Large quantities of sputum should be used.
2. Sputum should be diluted with sterile water.
3. Antiformin should be used in from 15-25 per cent solutions.
4. The sputum is dissolved in from 10-45 minutes—the bacilli, however, are uninjured after days of exposure in these solutions.
5. The resulting solutions should be watery in consistency and either colorless or pale yellow—if need be, more water or more antiformin may be added.
6. The tubercle bacilli may be obtained from the solution either by gravity sedimentation, by centrifugalization, or by the use of ligroin. Sedimentation may be facilitated by the addition of alcohol to lower the specific gravity. Of these several methods, gravity sedimentation is perhaps most used.
7. Washing of the sediment is unnecessary if egg albumin, diluted with 10 parts of water, plus 1 per cent formaldehyde; or some of the original sputum be used to fix the smear.
8. The smear stains perfectly by the Ziehl-Neelsen method.
9. In examining the smears one may find acid-fast rods which are to be differentiated from the tubercle bacillus by being larger, and straighter, and especially by the fact that they readily transmit light. These rods are probably fat needles, as suggested by Goerres.
10. In the entire smear there may be only two or three bacilli—but these are sufficient for a positive diagnosis.
11. Finally, by the use of antiformin in the examinations of sputum for tubercle bacilli one is enabled to demonstrate them in from 7.3 to 17.2 per cent of the specimens reported as negative after careful smear examination by the old method. This may be seen in the following reports:

Meyer (*loc. cit.*) in examining 113 sputa, found 14 positive by the ordinary smear examination. Of the remaining 99 so-called negative specimens, 14 or 14.1 per cent were demonstrated to contain tubercle bacilli by the antiformin method.

Haserodt (*loc. cit.*), in 340 examinations, classed 300 as negative after careful examination by the ordinary method. By the antiformin method 22 or 7.3 per cent of these negative sputa were shown to contain tubercle bacilli.

Goerres (*loc. cit.*), in 296 examinations by the ordinary method, found 162 negative; 28 or 17.2 per cent of these were definitely positive by the antiformin method.

Hüne (*loc. cit.*) reports 4 cases, repeatedly negative by the old method, proved positive by the new method.

Some months ago the writer undertook a study of the antiformin method of sputum examination to determine its value and limitations.

After trying various procedures, the following was finally adopted as the most satisfactory both as regards method and results.

1. Place the entire 24 hours' sputum in a conical settling glass; if the amount be excessive it is perhaps better to use only 15-20 cc.
2. If the specimen is thick, add an equal volume of distilled water. Less tenacious specimens do not require so much dilution.*
3. Add an amount of antiformin equal to one-fourth the volume of the diluted sputum; in other words, sufficient to make a 20 per cent solution.
4. Stir thoroughly, thereby breaking up the masses of mucus and greatly hastening complete solution.
5. Allow to stand till solution appears homogeneous. It should now be watery in consistency and pale yellow in color; if necessary, more water or more antiformin should be added and digestion allowed to continue. This will usually require from a few minutes to an hour, but may be allowed to continue for days with no resulting harm to the tubercle bacilli.
6. Add an equal volume of 95 per cent alcohol. By this procedure the specific gravity is reduced from about 1.030 to below 1.000; thereby not only hastening sedimentation, but making it more complete.
7. After stirring, allow to stand till sedimentation is complete. This will occur in 2-4 hours, but a period of 12-24 hours is recommended. During this sedimentation it may be necessary to gently turn the vessel to dislodge little particles of sediment which may be adhering to the sides of the vessel.
8. Pour off this clear supernatant fluid.
9. Make smear from the sediment on a glass slide, using some of the original sputum to aid in fixing the smear. This is best done by making a smear from the sputum before antiformin is added and afterwards spreading the sediment from the sputum-antiformin mixture on the same slide. Stain and examine in the usual way.

If one has the proper laboratory facilities the centrifuge may be used to hasten the work, or to remove the last trace of doubt as to the accuracy of the results; but from the reports of the other observers and my own exeprience, if tubercle bacilli are present, they will appear in the sediment obtained after adding alcohol. The use of the centrifuge merely shortens the time element, but in so doing adds much to the difficulties of the method.

Antiformin has been used in the examination of 103 sputa. Twenty specimens were obtained from the patients at Eudowood Sanitarium through the courtesy of Dr. Sloan, three from the Epstein Hospital, through the courtesy of Dr. Grover, and the remaining from the patients in attendance at the Phipps Dispensary of the Johns Hopkins Hospital. For the

* Here it might be well to make sure that the distilled water harbors no acid-fast organisms.

collection of the sputum wide-mouthed bottles of 100 cc. capacity were used, these being carefully washed in distilled water before being distributed. The patients were instructed to expectorate directly into the bottles, beginning with the first morning expectoration and continuing to use the bottles for the next 24 hours, or until they had accumulated from 15-20 cc. of sputum. However be it here noted that in this regard the results were very disappointing, as the dispensary patients frequently returned the bottles with but two or three cubic centimeters of sputum and in many cases this was without doubt entirely from the upper air passages. The results from the sanitaria were more satisfactory.

Dividing the cases examined according to the clinical diagnosis, 73 were definite pulmonary tuberculosis, 6 were probable pulmonary tuberculosis, 16 were doubtful pulmonary tuberculosis and 8 were definitely non-tuberculous.

By the old method all the sputa of the non-tuberculous, doubtful and probable groups, 30 in number, were negative. Of the 73 clinically positive cases, 31 showed tubercle bacilli in the smears examined.

By the antiformin method the examination of the same specimens showed no tubercle bacilli in any of the non-tuberculous, doubtful or probable cases. In the 31 cases positive by the old method great enriching was seen. In the ordinary smear an average of 25 bacilli could be counted in two minutes. In the antiformin smear an average of 138 tubercle bacilli could be counted in the same space of time. Of the 42 remaining specimens from clinically positive cases which, by ordinary smear examination, were negative for the tubercle bacillus, seven or 16.6 per cent were demonstrated to contain the bacillus by the antiformin method; or considering all the positive, probable and doubtful cases, a total of 95; 64 were negative by the old method, but 10.9 per cent of these were demonstrated to contain tubercle bacilli by the antiformin method.

I therefore feel fully justified in heartily recommending the use of antiformin in the examination of sputum for tubercle bacilli, not only in hospitals and dispensaries, but in the laboratory of every private physician. The general adoption of this method, combined with repeated examinations, will do much towards reducing the percentage of cases clinically diagnosed as definite pulmonary tuberculosis, in which however an *absolute* diagnosis cannot be made. Of far greater value than the finding of tubercle bacilli in the sputum of cases clinically diagnosed as definite pulmonary tuberculosis, is the possibility which this method offers of demonstrating bacilli in the cases still classed as only probable or doubtful, thereby enabling us to enforce a vigorous and systematic treatment at a time when such treatment is of most avail.

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# SERUM TREATMENT OF HEMORRHAGIC DISEASES.

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Under the designation "Hemorrhagic Diseases" is grouped a number of widely different morbid conditions, having hemorrhage as a common feature. The bleeding varies in location and in severity from insignificant cutaneous hemorrhages in mild cases of purpura, to appalling and even fatal losses of blood in more serious conditions.

An enumeration of the diseases with which hemorrhage is, or may be associated and which are sometimes designated as hemorrhagic diseases includes:

Hemophilia, hereditary and spontaneous.

Hemorrhagic diseases of the new-born, of which there are doubtless several forms.

Purpuras, acute, chronic, simple, rheumatic, senile, etc.

Jaundice.

Grave anemias.

A heterogeneous group, including nephritis, typhoid, and other severe infections.

Is hemorrhage in these conditions dependent upon a common cause or are different factors operative? At present we are unable satisfactorily to classify hemorrhagic diseases as we do not know the causes of the hemorrhagic diathesis, and in order to study this subject intelligently, a consideration of the theories of the coagulation of normal blood is necessary. There are almost as many theories concerning coagulation as there are investigators in this field. If we content ourselves with stating the points upon which all are agreed our task is easy. The coagulation of the blood depends upon the action of fibrin ferment, which normally is formed only after the blood is shed, on fibrinogen, which exists as such in the circulating blood. This much is fairly simple. Fibrinogen is a

protein body which forms 0.22-0.4 per cent of the plasma from which it can be obtained in approximately pure state, and its characteristics are fairly well determined. The part it plays in coagulation is known, but since hemorrhage is a pathological condition and coagulation does not take place under normal conditions, this action of fibrinogen can scarcely be taken as representing a normal function. Concerning its origin, the use to which it is put under normal conditions, and its fate in the body, we know little with certainty. The origin and mode of action of fibrin ferment is enveloped in still greater obscurity, although something is known of the finished product itself.

The term fibrin ferment is now used less than formerly, as the ferment nature of this body has been seriously called into question. Thrombin, as it was designated by A. Schmidt, has been isolated in approximately pure form and its properties studied by Howell.[1] Thrombin added to a solution of fibrinogen, causes coagulation by the formation of fibrin. Thus we have as factors in the much studied coagulation of the blood two substances, fibrinogen and thrombin which together form fibrin, the essential feature of the blood clot. Not only is the origin of thrombin the subject of many conflicting theories, but also the mode of action of thrombin on fibrinogen in the production of fibrin. Whether it is a ferment, a chemical, or a physico-chemical action is still undetermined.

Thrombin does not exist as such in the circulating blood, but since normally it appears within a few minutes after the blood is shed, it seems evident that all of the constituents which enter into its formation must be present in the blood before it is shed. Most theories concerning coagulation postulate, therefore, as a normal constituent of the blood, a mother substance of thrombin, usually designated prothrombin or thrombogen. While there can scarcely be a doubt as to the existence of prothrombin, it has never been identified or isolated. To account for the conversion of prothrombin into the active substance, thrombin, the presence of a ferment has been assumed, and to this ferment Morawitz gives the name thrombokinase. Since it has been found that blood does not coagulate if the calcium which it contains is precipitated in insoluble form as soon as the blood is shed, soluble calcium salts have been recognized as essential to the action of thrombokinase in converting prothrombin into thrombin. This kinase has never been isolated and its existence is really a matter of theory. Calcium salts of course do exist in the blood and their presence can be quantitatively determined, but the exact part they play in coagulation remains a subject of controversy.

Other substances which have been postulated in various theories to account for the coagulation of the blood, such as thrombozym, zymoplastic substances, etc., need not be considered here, but it is necessary, for the purpose of this paper, to go one step further into the theories concerning the formation of thrombin. We may accept as a necessary part of any theory, the existence of prothrombin normally in the circulating blood. Something happens when the blood is shed which causes the prothrombin to pass over into thrombin and since we have no more satisfactory explanation of this transformation we may refer it to the action of a kinase. But what is the source of kinase? There are numerous theories on this subject. There is one change in the blood which we know begins to take place as soon as it is shed, and this is the disintegration of the platelets, and less rapidly, the disintegration of the leucocytes. It is not unnatural, therefore, that investigators have sought the origin of thrombokinase in these elements.

We have attempted to develop from the literature, a more or less general and somewhat composite theory of coagulation. It serves to illustrate the highly speculative character of much that is held concerning this important phenomenon and indicates the difficulties to be overcome in arriving at the etiology and classification of the hemorrhagic diseases.

Hemophilia is the most striking and most interesting disease in this group and it is natural that it should have been most studied. To account for the hemorrhages many theories have been advanced. These vary from such simple explanations as abnormal thinness of the blood vessel walls, leading to rupture under slight provocation; abnormally high blood pressure; a disproportion between the total amount of blood and the total capacity of the vascular system; to such profound and obscure explanations as that "in hemophilia we have to do with an inherited chemical (fermentative) degeneration of the protoplasm of the formed elements of the blood, and perhaps of the whole organism" (Morawitz and Lossen[2]).

It is not necessary to enter into a discussion of the various explanations which have been offered for the hemorrhage in hemophilia and other hemorrhagic diseases. Some have thought that there is a lack of one or more of the factors normally present and concerned in bringing about coagulation; others have considered that there are present in the blood of these patients abnormal substances which are antagonistic to coagulation. Thus a lack of fibrinogen, of available calcium salts, of prothrombin, of thrombokinase, or of the elements from which thrombokinase is supposed to arise, on the one hand, and antithrombin and other anticoagulants on the other hand, have been held responsible for the failure of the blood to coagulate. Thus it may be seen that until more exact information is obtained as to the cause of the hemorrhagic diathesis any measure directed towards its treatment other than mechanical must be largely empirical.

When one comes to consider hemostatic measures one finds a long list for disposal. Excluding the direct measures, ligation, tamponading, etc., which are applicable to accessible hemorrhages in normal individuals, less successfully oftentimes in those with a hemorrhagic diathesis, we have the following: administration of calcium salts to increase the coagulability of the blood; injection of gelatin to increase its viscosity; administration of adrenalin to narrow the vessels. Unfortunately, the latter drug raises blood pressure, unless applied merely locally and while the local application does sometimes arrest hemorrhage, its use is accompanied by the danger, that if the hemorrhage does not cease entirely during the time the vessels are constricted, it is apt to become more profuse upon the subsequent dilatation, which occurs after the constricting action passes off.

Ergot probably has little value as a hemostatic aside from its use in obstetrics.

Other drugs which are classed as hemostatic are ferric chloride, silver nitrate, hydrastin, hydrastinine, gallic acid, lead acetate, etc. None of these, however, have proven very successful in the conditions under discussion.

Recently the treatment of hemorrhagic diseases has attracted renewed attention. It is to the investigations of Weil that the present trend of the work is usually ascribed. In 1905 he published his investigations concerning the pathogenesis and serotherapy of hemophilia,[3] which have been followed by numerous papers by himself and others.[4, 5, 6, 7, 8, 9]

Weil's work may be very briefly summarized as follows: The hereditary and spontaneous forms of hemophilia can be differentiated in origin, severity of clinical manifestations, character of the blood and its mode of coagulation. The faulty coagulation in the hereditary form is referred to the presence in the blood of anticoagulants; in the sporadic form, to the absence of substances normally present and essential to coagulation. He considers the changes of the blood in marked hemorrhagic conditions as analogous to, but not identical with, those of hemophilia and recommends, as a cure for the sporadic type of hemophilia and for many of the other hemorrhagic states and as the best therapeutic agent available in the treatment of hereditary hemophilia, subcutaneous injections of 30 cc. or intravenous injections of 15 cc. fresh human or animal serum. Eliçagaray [10] confirms Weil's work.

Baum [11] repeated much of Weil's experimental work, most of which he was unable to confirm. In reviewing his experience he states that he was much encouraged by his first case in which local applications of serum apparently stopped the hemorrhage, following the extraction of a tooth, in a hemophilic. In his succeeding cases, however, and in his animal experiments local applications of serum seemed entirely without effect. As to injections he found that in hirudinized rabbits, the coagulation time was much reduced by injections of serum, except in cases in which the blood had been rendered incoagulable to a marked degree by hirudin.

Baum treated three cases of hereditary hemophilia by means of serum injections with negative results, but thinks that in other hemorrhagic conditions good results may follow its administration.

Trembur [12] reports one case of hemophilia successfully treated by injections of sheep and rabbit serum.

Lommel [13] reports the successful treatment of hemorrhages in a four-year old hemophilic by the use of subcutaneous injections and local applications of antistreptococcic serum.

Class [14] reports one case of purpura hemorrhagica, which he considered cured by injections of antidiphtheritic serum.

Leary [15] reports a series of twenty cases treated by means of serum injections. His cases may be summarized as follows:

1. Jaundice, nine cases. The first case did not receive serum until after the hemorrhage had set in, following an operation. This patient died. Each of the remaining eight cases received before operation, as a prophylactic measure, 30 cc. rabbit serum subcutaneously. All but one of these recovered with but little bleeding following the operation. In the fatal case the author ascribes the death not to hemorrhage but to cholemia.

2. Hemorrhage of the new-born, three cases, two recoveries, one death.

3. Hemophilia, one case received diphtheria antitoxin, rabbit serum, and finally direct transfusion of human blood. Patient regained health rapidly.

4. Purpura, one case, cured.

5. Post operative hemorrhage, two cases, one cured, one died.

6. Uterine hemorrhage, one case, cured.

7. Typhoid fever hemorrhage, two cases, cured.

8. Ruptured tubal pregnancy (operated) one case, cured.

Among others who speak favorably of the use of serum injections in the treatment of hemorrhagic diseases is Welch,[16] who reports twelve cases of hemorrhage in the new-born, all of which he claims were cured by subcutaneous injections of fresh human serum. He injected as much as 209 cc. in five days, but averaged about 80 cc., in 10 cc. doses, extending over four days.

Schockaert [17] used subcutaneous injections of antistreptococcic serum successfully in two cases. One was a very anemic patient who bled profusely under operation. Fifty cubic centimeters of serum were injected and the author reports that immediately the hemorrhage stopped, so that the operation could be concluded with a dry field of operation. The second case was one of rebellious menorrhagia which resisted hydrastis, ergot, stipticine, etc., but was controlled by injections of 10 cc. of antistreptococcic serum at the beginning of the menstrual periods.

Chabrol [18] reports the successful serum treatment of a case of rheumatic purpura accompanied by intestinal hemorrhage which resisted ordinary treatment, calcium chloride, gelatin, ergot, etc. He gave daily injections of 10 cc. diptheria antitoxin subcutaneously for five days.

Bigelow [19] reports successful treatment of three cases of hemorrhagic disease of the new-born. He states that two of the cases were moribund when treatment was instituted, calcium lactate and epinephrin having been tried without benefit. The hemorrhages ceased after subcutaneous injections of 5 cc. fresh rabbit serum.

Busse [20] reports the use of fresh human serum in the treatment of 10 cases of uterine hemorrhage. The dose employed was 10 cc. injected intragluteally. Five of his cases he regarded cured, four were still under treatment at the time of his report.

A smaller number of reports have appeared in which the value of serum injections in hemorrhagic disease is denied. The authors of these reports usually advocate the use of injections of defibrinated blood instead of serum or else the direct transfusion of human blood. Thus Schwartz and Ottenberg [21] report the case of a child 11 days old with melena, hematemesis, hemorrhages from the nose and mouth, icterus and ecchymoses. One injection of horse serum (not fresh)

had no effect in stopping the bleeding. The child was therefore transfused from its father and the hemorrhages are reported to have immediately stopped. After some days, however, bleeding recurred and the patient died.

Mosenthal [22] reports a case of melena neonatorum cured by direct transfusion.

Swain, Jackson and Murphy [23] report a case of melena neonatorum unbenefited by 75 cc. of fresh rabbit serum subcutaneously and an injection of diphtheria antitoxin. Direct transfusion from the child's father was followed by complete cessation of the hemorrhage.

Lambert [24] reports a case of melena neonatorum in which transfusion was performed after the hemorrhage had been going on for three days and the child was in a dying condition. An immediate and permanent cure is said to have resulted.

Murphy [25] reports the case of a woman 48 years old, who for 17 years suffered attacks of abdominal pain, jaundice, clay colored stools and finally a very severe attack accompanied by hemorrhages from the nose and rectum. Injection of 30 cc. horse serum was without effect. Injection of 30 cc. fresh rabbit serum seemed to control the hemorrhages to a degree, but still the bleeding continued to an alarming extent. Coagulation time 13 minutes (Brodie-Russell). The patient was then transfused, her husband being the donor. Twelve hours after transfusion the coagulation time was 3-4 minutes and on the following day the patient was successfully operated on for gall stones, and was cured.

Duke [26] considers that the tendency to uncontrollable bleeding in at least some of the hemorrhagic diseases is due to a diminished number or to an absence of blood platelets. He therefore advocates transfusion as the only means of supplying the deficit and reports several cases which he considers to have been greatly benefitted by this procedure.

From this review of the literature, which is by no means complete, it is apparent that any treatment of these alarming and oftentimes dangerous conditions can be only empirical, until the cause of the disorder is determined. Meanwhile the use of serum injections, the injections of defibrinated blood, and direct transfusion seem to have yielded better results than any other measures. As to the choice between these three procedures, doubtless direct transfusion would be the ideal method were it not for the technical difficulties.

As to the intravenous introduction of defibrinated blood or foreign serum, one perhaps must feel some apprehension of danger, but the condition to be combated is a dangerous one and justifies, in the present state of our knowledge, the risk we may run in carrying out these procedures. Moreover, theoretically the danger from agglutination in transfusion is perhaps as great as the danger from thrombosis or hemolysis, following the injection of defibrinated blood or foreign serum. So far as we are aware no untoward results of a serious nature have been reported to follow the latter two measures. Finally, while it may be true that certain of the hemorrhagic diseases depend upon a lack of blood platelets and that this deficit can only be supplied by direct transfusion, still it is more probable that it is not the platelets *per se* which contribute towards the checking of hemorrhage, but products which are set free on their disintegration. These products are doubtless furnished just as well in serum or defibrinated blood as in the whole blood. It would seem that injections of serum or of defibrinated blood would answer as well as direct transfusion in all cases except those—if clinically there are such—which depend upon a lack of fibrinogen.

A consideration of great practical importance is, that in the case of injections of serum or of defibrinated blood one knows certainly that he gets the injection into the patient and one can accurately gauge the amount introduced, while in transfusion, except in the hands of an expert surgeon, there is much uncertainty of transferring any blood at all, and in no case can the amount transferred be satisfactorily determined.

Our series consists of six cases admitted to the wards of the Johns Hopkins Hospital.* The number is small and no conclusions should be drawn from them, but the results were so encouraging that the method seems to deserve further consideration.

In every case the serum or defibrinated blood was used within 2-3 hours after it was drawn, except in Case 4 where normal horse serum was used.

CASE 1.—*Typhoid Fever.* Male, age 31, black, single, laborer. Admitted August 6, 1910.

*Complaint:* Headache and pain in back.

*Family History:* Not important.

*Past History:* Measles at 21; hard work as laborer; alcohol, weekly sprees.

*Present Illness:* Onset two weeks before admission,—headache and fever, also diarrhœa.

*Physical Examination:*. Well-nourished, muscular man. Tongue coated; general glandular enlargement; spleen palpable; sputum contained some blood clots. Hæmoglobin 100 per cent.

August 17: Bleeding from gums. Silver nitrate adrenalin chloride and calcium lactate without effect.

August 18: Ecchymoses appeared on conjunctiva and purpuric spots on abdomen. Hematuria. Red blood cells 2,912,000. Hæmoglobin 80 per cent.

August 19: Feces continue to contain blood; 13 cc. defibrinated human blood injected intravenously, followed by headache and restlessness.

August 20: 13 cc. defibrinated human blood, intravenously.

August 21: 17 cc. defibrinated human blood, intravenously.

August 22: 12 cc. defibrinated human blood, intravenously.

Following these injections the bleeding seemed less profuse but the blood count continued to fall and showed on the latter date, red blood cells 1,448,000. Hæmoglobin, 35 per cent.

August 23: In view of patient's desperate condition, 320 cc. of defibrinated human blood from a case of polycythemia was given intravenously. This was followed by headache, chill, extreme abdominal pain and rapid pulse.

August 24: Red blood cells 2,880,000. Hæmoglobin 40 per cent. Patient feeling much better but still slight oozing from gums and blood in his stools. 250 cc. defibrinated human blood was injected intravenously, followed by severe shaking chill with elevation of temperature and pulse.

August 26: 20 cc. defibrinated human blood intravenously.

August 27: 200 cc. defibrinated human blood intravenously.

* We are indebted to Drs. Halsted, Barker and Thayer for permission to report these cases.

This injection was well borne, there being no chill or elevation of temperature after it. From this date convalescence was rapid, and no further hemorrhages occurred.

September 26: Red blood cells 3,344,000. Hæmoglobin 72 per cent. Patient discharged well.

In this case two serious conditions presented themselves, one resulting from the other; persistent hemorrhage and grave anemia. Large injections of defibrinated blood were used in this case rather than small injections of serum with the hope that the fibrin ferment introduced would check the hemorrhages and that the foreign red blood cells might continue their normal function in our patient and thereby relieve his anemia. The blood counts before and after the first large injection of defibrinated blood seem to indicate that this hope was realized.

It was considered by all those who observed this case that the injections of defibrinated blood exerted a favorable influence and were perhaps the means of preventing a fatal issue.

Case 2.—*Chronic Nephritis, Myocarditis, Uremia.* Male, white, aged 43, married, lumber merchant. Admitted October 13, 1910.

*Complaint:* Weakness, stupor and sour stomach.

*Family History:* Father dead, aged 73, heart trouble; mother, four brothers and one sister, living and well. Three sisters died of tuberculosis about 25 years ago. Married seven years, no children.

*Past History:* Whooping cough at 14, measles and gonorrhœa at 17. At 36 a diagnosis of tuberculosis was made; patient later was pronounced cured. Malaria at 41. Patient has used alcohol and tobacco until recently, two cigars and two or three drinks daily. Has done hard physical work with much exposure all his life.

*Present Illness:* Onset six months ago. Started with feeling of weakness, dullness and headache. Within twenty-four hours vomiting, delirium, convulsions. After two weeks patient was well enough to return to work, and kept about his business until one week before admission to the hospital. At this time (October 4) again had headache, vomiting and convulsions. Since the first admission, six months ago, patient states that he has been subject to headache, increased urination, dyspnœa, œdema of ankles and impairment of vision.

*Physical Examination:* Sparely nourished man, dull, urinous odor to breath, vision impaired. Systolic murmur at apex and over body of heart, transmitted to axilla. Well marked arteriosclerosis.

October 14: Red blood cells 3,100,000. White blood cells 16,800. Hæmoglobin 50 per cent.

October 15: Five hemorrhages from bowels; prior to this date stools contained no blood.

October 16: Five hemorrhages from bowels. Following the last hemorrhage 30 cc. of fresh rabbit serum was injected subcutaneously into the thighs. Following this injection, there was no further bleeding.

October 17: Red blood cells 1,700,000.

October 20: Patient sank into a deep coma and died.

Although this patient died, it is to be noted that there was an interval of four days between the injection of serum and death, during which no hemorrhage occurred.

Case 3.—*Cirrhosis of Liver (atrophic) and Hematemesis.* Male, white, aged 52, married, banker. Admitted October 19, 1910.

*Complaint:* Weakness, swelling of feet, and vomiting of blood in February, 1910.

*Family History:* Not important.

*Past History:* Measles as a child, typhoid at 37, recovery good. Very closely confined to business, takes little exercise; 6 to 10 cigars daily. Hearty eater. No alcohol. Constipation for past two years.

*Present Illness:* Eight months ago, when as well as usual, was taken suddenly with a feeling of weakness and vomiting of blood, passing considerable amount of blackish material from the rectum. Remained at home for one week and was in hospital for three weeks. Shortly after returning to business was constipated and had a fecal impaction removed by physician, after which for a time he enjoyed a feeling of perfect health. Several months prior to his present hospital admission he had an attack of indigestion accompanied by dizziness and vomiting at intervals of six or eight hours. Vomitus was at first blood stained.

*Physical Examination:* Large fleshy man, pale, skin slightly yellow, heart enlarged, loud systolic blow over precordium and in axilla. Red blood cells 1,846,000. White blood cells 5000. Hæmoglobin 39 per cent.

November 2: Patient suffered three attacks of hematemesis; was given morphine and calcium lactate.

November 3: Three attacks of hematemesis, vomiting approximately one liter of blood. Following the last hematemesis 30 cc. of fresh rabbit serum was injected subcutaneously.

November 4: Gradual onset of weakness, unconsciousness, and death.

There was no further evidence of hemorrhage after the administration of serum, but at autopsy the stomach was found to contain a large amount of dark blood.

Case 4.—*Chronic Pancreatitis, Jaundice, Exploratory Laparotomy, Drainage of Gall Bladder.* Male, white, aged 30, clerk. Admitted to hospital October 20, 1910.

*Complaint:* Sick stomach and jaundice.

*Family History:* Not important.

*Past History:* Generally healthy; measles in childhood.

*Present Illness:* Onset, according to patient, ten years ago. At this time he had pain in the right kidney region after any violent exercise and associated with this was severe pain in the right testis. Four years prior to admission had attack of severe abdominal pain, accompanied by nausea but no vomiting. These attacks recurred at intervals for two years; jaundice for the past year.

*Physical Examination:* Negative except for jaundice.

October 26. Exploratory operation for obstructive jaundice Chronic pancreatitis was found, the head of the pancreas obstructing the common duct. Drainage of gall bladder, no stones.

November 7: Eleven days after operation, patient began to bleed from the wound in the abdominal wall. Hemorrhage could not be controlled by the ordinary means. 20 cc. of normal horse serum (less than one month old) was injected intravenously, followed by chill and sharp elevation of temperature. There was no further hemorrhage, however, and patient subsequently made a satisfactory recovery from his operation.

This is perhaps the most striking case in the series. The extremely obstinate nature of the hemorrhages in jaundice cases is well known and so alarmed were the surgeons in charge of this patient that they were unwilling that he should wait the two or three hours necessary for obtaining perfectly fresh rabit serum.

The hemorrhage ceased after a single injection of normal horse serum and it seems justifiable perhaps to believe that the serum stood in a causative relation to this result.

Case 5.—*Typhoid Fever.* Male, white, aged 17, single. Admitted to the hospital November 12, 1910.

*Complaint:* Fever and pain in stomach.

*Family History:* Not important.

*Past History:* Always healthy. Seven months prior to admission operation for appendicitis.

*Present Illness:* Began six weeks before admission; headache, chills and anorexia.

*Physical Examination:* Sparely developed and undernourished, tongue coated. Spleen palpable and soft. Rose spots present. (Blood culture *B. typhosus.*) Red blood cells 4,900,000. White blood cells 6000. Hæmoglobin 75 per cent.

November 16: Red blood cells 4,600,000. White blood cells 3900. Hæmoglobin 63 per cent.

November 23. Intestinal hemorrhages aggregating approximately 1750 cc.

November 25: 15 cc. fresh rabbit serum intravenously. Since then there have been no further hemorrhages.

CASE 6.—*Typhoid Fever.* Male, white, aged 21, single. Admitted to hospital on October 4, 1910.

*Complaint:* Headache.

*Family History:* Not important.

*Past History:* Mumps with double orchitis, measles; otitis several times five years ago. Gonorrhœa one year ago. Tobacco and alcohol very rarely. Works in open air, exposed to weather.

*Present Illness:* Onset, with drowsiness ten days before admission; six days before admission pain in stomach, headache, soreness in legs, feverish, anorexia, cough and diarrhœa.

*Physical Examination:* Well nourished man, tongue coated, palpable spleen, rose spots. Red blood cells 6,148,000. White blood cells 5080. Hæmoglobin 98 per cent.

October 11: There were four intestinal hemorrhages, aggregating about 500 cc.

October 14: Hemorrhages continued and it is estimated that the total loss of blood amounts to approximately 2000 cc. On this date patient received 30 cc. of fresh rabbit serum, subcutaneously.

October 15—6 a. m.: About 75 cc. of blood was passed from rectum, but the patient's condition was much improved. 11 a. m, 30 cc. fresh rabbit serum injected subcutaneously. 8 p. m., about 100 cc. of dark blood was passed from rectum. Following this there was no further hemorrhage, but on October 19, patient showed signs of perforation and at operation an opening was found in the ileum through which fecal matter exuded.

October 23: Patient died of acute broncho-pneumonia.

In this case on the day following the first injection of serum there was approximately 175 cc. dark blood passed per rectum. It is possible that this was blood which had been shed into the intestine before the serum injection. The subsequent perforation (four days after) and death (eight days later) of the patient does not detract from the idea that the serum injections were successful in combating the hemorrhages.

The series of cases here reported is small and in several instances a fatal termination ensued, but it is to be remembered that the serum injections were not given in the hope of curing typhoid fever, nephritis, cirrhosis of the liver, etc., but to combat the hemorrhage which was associated with these conditions. If the hemorrhage was stopped by the use of serum injections, even though the patient eventually died, our hopes were fulfilled.

In our present state of knowledge, or lack of knowledge, concerning the causes of hemorrhages in these various conditions, the treatment is necessarily empirical. The most plausible explanation of any good which injections of serum or defibrinated blood may do is that fibrin ferment is thereby introduced into the circulation.

Since it is known that fibrin ferment (thrombin) readily passes over into an inactive form (meta-thrombin) on standing, it seems advisable to use serum or defibrinated blood as fresh as possible.

We have used rabit serum because it is easily obtained in a fresh condition, is but slightly, if at all, toxic for human beings, and if sensitization of the patient follows, the danger is not very great, since it is unlikely that subsequent administrations of rabbit serum would take place.

If horse serum is used one has to consider very carefully if the patient may not have been sensitized by a previous injection of antitoxin (horse serum) or may not be sensitized to a subsequent injection of antitoxin.

As to choice of methods, it seems advisable to inject directly into the circulation, although in so doing the possibility of causing intravascular clotting must be borne in mind.

If the patient has already been rendered anemic by serious hemorrhage, injection of large amounts of defibrinated blood directly into the circulation may not only check the hemorrhage but combat the anemia. With this end in view human blood should be used, since the red cells from one species cannot functionate in a different species, and it is likely that they would be quickly hemolyzed and give rise to toxic symptoms. In order to insure the best chance of success the donor should be a person from the same group (according to the isoagglutination reaction)[11] as that to which the patient belongs, thus avoiding possible danger from isoagglutination and isohemolysis.

In view of the unsatisfactory results attending the use of the ordinary methods of treating the hemorrhage in these conditions and the encouraging results from injections of serum and defibrinated blood reported here and elsewhere, we believe the later procedures are deserving of a more extensive trial.

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# CONCERNING THE MUCH-HOLZMANN REACTION.

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Based upon the investigation of a considerable series of cases Much & Holzmann[1] proposed a biological test for the diagnosis of certain forms of insanity (dementia precox and maniacal depressive insanity) which they designate as the "psycho-reaction."

The reaction consists, according to the originators, in the ability of the serum from patients suffering from the two forms of insanity mentioned, to inhibit the hemolysis of normal red blood cells by cobra-venom; the serum from patients with other forms of insanity (epilepsy with circular disturbances excepted) and from normal individuals having no such action.

The authors carried out the test on the serum of 400 individuals and state that 100 per cent of all cases in which a diagnosis of dementia precox or maniacal depressive insanity could be established with certainty, gave a positive "psycho-reaction."

Much[2] reports the results of his investigation on additional cases, confirming his previous claims and considers the reaction dependent upon a quantitative or qualitative disturbance in the cholesterin content of the serum, due to altered metabolism indicating an inherited predisposition.

Bauer[3] objects to the term "psycho-reaction," as he does not consider the reaction a specific one.

He investigated the action of the serum from umbilical cord blood in 17 cases and found, that in the presence of 14 of these, no hemolysis of red corpuscles by cobra-venom took place, while in one case, only a trace of hemolysis occurred, and in the other two cases the hemolysis was partial.

In the presence of the serum of 16 older children and adults, the hemolysis was either partial or complete in every case; while in the presence of the serum of nine nursing infants no hemolysis occurred in one case, and was either partial or complete in the remaining eight.

Bauer thinks that the explantion of these results is to be sought in a paucity of activating lipoids in the serum (perhaps lecithin) rather, than in an increase of inhibiting substances (cholesterin).

Huebner and Selter,[4] using the same technic as Much and Holzmann, state the following results, which were the outcome of experiments with 82 cases of dementia precox, maniacal depressive insanity and other types of functional and organic psychoses and neuroses.

1. The cobra-venom reaction was found not only in cases of maniacal depressive insanity and dementia precox, but also in many other diseases, psychoses as well as neuroses and organic brain and spinal cord diseases.

2. The reaction was positive in less than 50 per cent of the positively diagnosed cases of maniacal depressive insanity and dementia precox.

Hirschl and Pötzl[5] found in a number of cases of the hebephrenic and catatonic forms of dementia precox, an increased resistance of the erythrocytes and an increased inhibiting action of the serum against cobra-venom hemolysis. They reserve their opinion as to the clinical value of the test.

Beyer and Wittneben[6] examined the blood of 345 individuals with reference to the Much-Holzmann reaction. Their results are given in the following table:

Positive reactions occurred in the following cases:

I.

| | | |
|---|---|---|
| Dementia precox | 50% | = 50% |
| Maniacal depressive insanity | 31 | |
| Epilepsy | 60 | |
| Idiocy | 74 | |
| Other psychoses | 19 | |

II.

Somatic diseases ..... 25%

III.

Normal ..... 10%

from which they conclude that the reaction is of no differential diagnostic value. It appears to them rather as an expression of certain changes in metabolism, which can occur in the most different conditions, the exact mechanism of which, however, is unknown.

Fraenkel, Kathe and Bierotte[7] carried out the reaction in 92 cases, 58 of which were various types of psychoses, 26 were patients suffering from other than mental diseases. In 50 per cent of the psychoses a positive reaction was obtained, 23 per cent of the patients suffering from other diseases gave a positive reaction, while of eight normal individuals tested all gave a negative reaction; from which they conclude, that the reaction is not specific as claimed by the originators, but does differentiate between healthy and diseased individuals. They consider the reaction a quantitative rather than a qualitative one, since they found that all sera exert some inhibiting action. Differences in the resistance of the corpuscles of individuals were noted and in some cases, a given serum, when tested again one set of corpuscles, gave a positive reaction and when tested against other corpuscles gave a negative reaction.

No difference was found between active and inactivated serum. Controls were made to determine if the corpuscles were hemolyzed by the serum alone in any case (isohemolysis).

The authors consider that the Much-Holzmann reaction depends upon the relation between activating and inhibiting substances present in the serum. These bodies probably being lecithin and cholesterin.

Zalozieki[8] obtained the positive reaction, according to Much and Holzmann, in a number of different psychoses, as well as in normal individuals and almost constantly in the blood of the newborn. He considers the reaction diagnostically valueless and thinks that it depends upon the degree of muscular activity.

Schultz[9] tested the reaction in 151 cases and obtained a positive result in 50 per cent of all cases of dementia precox and maniacal depressive insanity. He also obtained positive results in a number of other psychoses and in some normal controls. A large number of his cases fell in the doubtful group, due to the difficulty of making a clear reading. Schultz could not find that various factors such as age, sex, temperature, pregnancy, general blood condition and state of nutrition were of any importance. The quantitative nature of the reaction is emphasized and he concludes that the reaction is of no diagnostic value. The relative amount of cholesterin and lecithin present, according to Schultz, will determine the outcome of the test.

Geissler[10] examined 500 cases and points out that it is necessary to select the time for testing the serum according to the clinical state of the patient, as the reaction is generally negative in the intervals between attacks of maniacal depressive insanity and is more often present in those phases of dementia precox associated with periods of definite excitement or depression. Using these precautions he obtained a positive reaction in all cases of dementia precox and maniacal depressive insanity, but found positive results in other types of psychoses, as well as in non-psychic diseases. Geissler did not confirm Much and Holzmann's claims as to the positive reactions in cases of epilepsy and in those having a family history of dementia precox and maniacal depressive insanity. Eisner and Kronfeld[11] found the reaction positive in 57.8 per cent of 19 cases of dementia precox and in 50 per cent of eight cases of maniacal depressive insanity. Although they found about the same percentage of positive reactions in other psychoses and a somewhat smaller percentage of positive reactions in mentally sound individuals, they conclude that the reaction occurs with greater frequency in the psychoses as a class, than in other states. As to the diagnostic value, they cannot confirm the work of Much and Holzmann.

Plaut[12] examined 21 cases in all and found a positive test in 38 per cent of the cases of maniacal depressive insanity, and in 20 per cent of the dementia precox group. He concludes that the reaction is of no diagnostic value as proposed.

Brueckner and Much,[13] after the study of 200 more cases, conclude that although the reaction is of great theoretical serologic interest, it is not of use in the diagnosis of dementia precox and maniacal depressive insanity as originally claimed. They point out that before the reaction can be of use in psychiatry it will be necessary to determine the nature of the inhibiting substances, the factors concerned in their appearance in the blood serum, and the apparent relation existing between their higher prevalence in mental diseases than in all others examined so far. They offer no solution.

Rosanoff,[14] after the examination of 185 persons, concluded that all human sera possess some power of inhibiting the hemolytic action of cobra-venom, but that the degree of inhibition is not sufficiently raised or uniform in cases of dementia precox or maniacal depressive insanity to make the test of diagnostic value. A positive reaction was obtained in other psychoses and in normal individuals. The fact that so high a percentage of those suffering with dementia precox (57.9 per cent) gave a positive reaction, led Rosanoff to believe that a positive test might add weight to the diagnosis in certain doubtful cases, where there were other symptomatological factors leading to the conception of dementia precox. All 15 cases of maniacal depressive insanity gave negative results in Rosanoff's hands.

Pfoeringer and Landsberger[15] conclude that the cobra-venom reaction is not specific in the sense of Much and Holzmann. They consider it the expression of general changes in metabolism, especially in oxidation processes, in which the

red blood corpuscles play the most important rôle; at any rate, they do not consider this idea so hypothetical as the views of those who connect the reaction with processes in the central nervous system, which have to do with changes in the lecithin and cholesterin content of the blood.

Alt,[16] without giving any of the details of the work upon which his conclusions were based, says, that in the 50 instances, in which the reaction was tried, he was unable to obtain results which would in any way permit of conclusions in agreement with those of Much and Holzmann.

Omorokow [17] found that the Much-Holzmann reaction occurred more commonly in the psychoses as a class than it did in the surgical cases and mentally normal individuals examined by him.

Nitsche, Shlimpert and Dunzelt [18] in 198 cases, of which 105 were dementia precox and 28 maniacal depressive insanity, found positive tests in 54.2 per cent of the former and 14.2 per cent of the latter. The duration of the disease, age, sex, drugs and nutritional state apparently exert no influence on the outcome of the reaction. Opposed to Geissler [16] they can find no interdependence between the results and the stage or clinical phase of the disease in question. The reaction is not considered as diagnostic of either dementia precox or maniacal depressive insanity.

Raubitschek and v. Dungern [19] in the discussion of the report by Much (*loc. cit.*) indicated that they could find no regularity in the results of the test as applied and that they found positive results in a great number of conditions other than dementia precox and maniacal depressive insanity.

Stilling [20] investigated 50 cases of dementia precox, repeating the work of Hirschl and Pötzl and found the resistance of the corpuscles to hemolysis by cobra-venom increased in only 14 cases. Twenty-two cases gave a positive Much-Holzmann reaction, when tested against corpuscles from cases of dementia precox.

The author is unable to confirm the results of either Hirschl and Pötzl or Much and Holzmann.

Bonfiglio [21] at the end of his examination of 67 cases states that the reaction of Much and Holzmann is not specific for either maniacal depressive insanity or for dementia precox. In other details, his experience has been that of almost all others, who have repeated the work. In a second communication [22] he gives a review of the literature which has appeared on this subject during the past several months.

From the foregoing summary of the literature it appears that no diagnostic significance can be attached to the Much-Holzmann reaction. Our own conclusions on this point are in accordance with those of the majority of investigators who have repeated Much and Holzmann's work. We have, however, made certain additional observations which we think justify the publication of our results.

The points which we have tried to determine may be briefly stated as follows:

1. Does the serum from cases of dementia precox and maniacal depressive insanity inhibit cobra-venom hemolysis?

2. Does a given serum always give the same reaction when subjected to the Much-Holzmann test?

3. Is the result of the test influenced by the relation between the groups (as determined by the isoagglutination reaction) to which the serum and corpuscles belong?

4. Is there any difference between the action of fresh and inactivated serum on cobra-venom hemolysis?

5. Do corpuscles of different groups offer different degrees of resistance to hemolysis by cobra-venom?

6. Does serum favor or retard the hemolysis of human erythrocytes by cobra-venom?

*Technique.*—In the first series of cases we followed exactly the technique given by Much and Holzmann. The cobra-venom was obtained for us from Drs. Flexner and Noguchi of the Rockefeller Institute of New York by Dr. H. J. Berkley. It consisted of a 1 per cent solution in 50 per cent glycerine. This stock solution was kept in the ice chest and from it fresh dilutions were made for each series of tests.

From the first experiments it was evident that this venom had much greater hemolytic strength than that used by Much and Holzmann, so that after a few preliminary tests it was found necessary to use a 1-25000 dilution instead of a 1-5000 dilution as recommended by the originators. Later we further modified the technique in that we combined equal parts (0.25 cc.) of cobra-venom solution, serum, and a 5 per cent suspension of washed corpuscles, instead of 0.35 cc. serum, 0.25 cc. cobra-venom solution and 0.5 cc. of a 10 per cent suspension of corpuscles. The modified technique gave better results in our hands than that proposed by Much and Holzmann.

Considerable difficulty was experienced in reading the results of the test, as we got all grades of hemolysis from very slight up to complete, no matter how we varied the proportions of serum, corpuscles, and venom (the differences were as great in tests with normal serum as in those with serum from cases of dementia precox or maniacal depressive insanity), so that in our series a considerable number of cases fall into the group giving doubtful reactions.

We made no effort to collect a large series of cases, but on the other hand repeated the test a number of times with serum from the same cases on different days, and frequently put up parallel series, using corpuscles from a different person in each series.

We have tested the serum of 49 individuals with the following results:

TABLE I.

| Number of Cases | Condition | Positive reactions | Negative reactions | Doubtful reactions |
|---|---|---|---|---|
| 21 | Dementia precox ..... | 9 | 7 | 5 |
| 4 | Maniacal depressive insanity. | 2 | 2 | 0 |
| 8 | General paresis ..... | 1 | 6 | 1 |
| 16 | Sundry other psychoses..... | 0 | 6 | 0 |
| 10 | Diseases other than mental and normal ..... | 2 | 3 | 5 |

A number of the above cases were tested on two, three, four and five occasions, but without any reference to psychic states. The results for a few of these cases, chosen at random from a considerable number of cases in which repeated tests were

made; are given in Table II. They show that the reaction is fairly constant for a given individual, varying sometimes from doubtful to positive or from doubtful to negative, but only once, Case 6, from negative to positive.

TABLE II.

| Case Number | Oct. 19th 1909 | Oct. 27th 1909 | Nov. 5th 1909 | Nov. 8th 1909 | Nov. 17th 1909 | June 25th 1910 |
|---|---|---|---|---|---|---|
| 1 | — | — | ± | — | ± | |
| 2 | — | — | | | | |
| 3 | ± | + | + | + | ± | |
| 4 | ± | ± | ± | | | |
| 5 | — | | | | ± | |
| 6 | — | | + | + | ± | |
| 7 | — | | | | ± | |
| 8 | + | | + | + | + | |
| 9 | + | + | | | | |
| 10 | | | | + | + | + |

In performing the "psycho-reaction" Much and Holzmann recommend that the serum be tested against several different sets of corpuscles rather than against a single set.

It has been shown [23 24] that individuals may be divided into four groups according to the isoagglutinative action of the serum and corpuscles. Isohemolysins have long been known to exist normally in human serum and recently it has been shown [25] that the serum of every individual possesses an anti-hemolysin which will protect not only his own corpuscles from isohemolysis, but also those of any member of the group to which he belongs.

It occurred to us that perhaps human serum might have some such protective action as the above against cobra-venom hemolysis; that is, that a given serum might protect its own corpuscles and others belonging to the same group from hemolysis by cobra-venom, and that a positive Much-Holzmann reaction (inhibition of hemolysis) might occur when the serum tested came from a member of the same group as that to which the individual belonged whose corpuscles were used in the test, and that a negative reaction (hemolysis) might result, when the serum and corpuscles came from different groups.

We further thought that negative reactions might result in some cases from the well known isohemolytic action of certain sera.

In order to determine these two points we always determined the group to which the serum belonged and also tested to see if it possessed an isohemolysin for any of the corpuscles used, and in a considerable number of cases we set up parallel series of tubes so that each serum was tested against corpuscles from several different groups. A single series will serve to illustrate the results.

With regard to the groups to which the serum and corpuscles belonged, reference to Table III shows that, except for very minor variations in degree, the hemolysis proceeds equally well when the corpuscles and serum belong to the same group as when they are from different groups. Furthermore, corpuscles in the presence of their own serum are hemolyzed by cobra-venom to the same extent as in the presence of serum from another individual, provided the latter contains no isohemolysin for the corpuscles in question.

With regard to the effect of isohemolysis on the result of the Much-Holzmann reaction, we encountered but few sera in our series which were hemolytic for the corpuscles used, but as far as our observations go, they tend to show that if a given serum is tested against several sets of corpuscles and is found to be hemolytic for one set but not for the others, this serum may give a positive Much-Holzmann reaction when tested against the corpuscles for which it is not hemolytic and a negative reaction when tested against the corpuscles for which it is hemolytic.

TABLE III.

Showing the Relation Between the Groups to Which the Serum and Corpuscles Belong and Contrasting the Action of Active and Inactive Serum.

| Venom | Serum. No. | Serum. Gr. | Corps. Gr. | 2 hours at 37° C. Serum active | 2 hours at 37° C. Serum inactive | 22 hours in ice chest Serum active | 22 hours in ice chest Serum inactive |
|---|---|---|---|---|---|---|---|
| 0.25cc. | 20 | IV | II | gK | gK | L | L |
| " | " | " | III | gK | gK | L | L |
| " | " | " | IV | gK | K | L | fvL |
| " | 21 | II | II | fvH | gK | kK | kK |
| " | " | " | III | gK | gK | kK | kK |
| " | " | " | IV | fvH | gK | kK | kK |
| " | 22 | II | II | gK | gK | L | fvL |
| " | " | " | III | gK | gK | L | fvL |
| " | " | " | IV | gK | K | fvL | fvL |
| " | 23 | IV | II | gK | gK | kK | K |
| " | " | " | III | gK | gK | kK | K |
| " | " | " | IV | fvH | gK | kK | K |
| " | 24 | IV | II | K | K | L | L |
| " | " | " | III | K | K | L | L |
| " | " | " | IV | K | K | L | L |
| " | 25 | IV | II | fvH | gK | kK | kK |
| " | " | " | III | fvH | gK | kK | kK |
| " | " | " | IV | fvH | gK | kK | kK |
| " | 26 | II | II | gK | K | fvL | fvL |
| " | " | " | III | gK | gK | fvL | fvL |
| " | " | " | IV | fvH | K | fvL | fvL |
| " | 27 | II | II | fvH | gK | fvL | kK |
| " | " | " | III | fvH | gK | fvL | kK |
| " | " | " | IV | fvH | gK | fvL | kK |
| " | 28 | IV | II | K | gK | L | fvL |
| " | " | " | III | K | gK | L | fvL |
| " | " | " | IV | K | K | L | fvL |
| " | 29 | IV | II | K | K | L | L |
| " | " | " | III | K | K | L | L |
| " | " | " | IV | K | K | L | L |
| " | 30 | IV | II | gK | gK | fvL | kK |
| " | " | " | III | gK | gK | kK | kK |
| " | " | " | IV | gK | gK | kK | kK |
| " | 31 | IV | II | gK | gK | fvL | fvL |
| " | " | " | III | gK | gK | fvL | fvL |
| " | " | " | IV | gK | gK | fvL | fvL |
| " | 32 | II | II | gK | gK | fvL | fvL |
| " | " | " | III | gK | gK | L | fvL |
| " | " | " | IV | fvH | gK | fvL | fvL |
| " | 33 | III | II | fvH | fvH | kK | kK |
| " | " | " | III | H | fvH | kK | kK |
| " | " | " | IV | fvH | fvH | kK | kK |
| " | 34 | IV | II | gK | gK | fvL | fvL |
| " | " | " | III | gK | gK | fvL | fvL |
| " | " | " | IV | gK | K | fvL | fvL |

The grade of hemolysis is estimated by the color of the supernatant fluid and the amount of sediment in the tube.
H = no hemolysis. kK = small sediment.
fvH = trace hemolysis. fvL = almost complete solution.
gK = large sediment. L = complete solution.
K = sediment.
The corpuscles used in the above series were from cases Nos. 32, 33, 34.

In several series of experiments we contrasted the effect of active serum and serum inactivated at 56° C. for 30 minutes, on cobra-venom hemolysis. Table III gives one such series and shows that there was only a slight and inconstant difference between the two.

We could discover no difference in the resistance of corpuscles of different groups to hemolysis by cobra-venom and our experiments to determine if serum favored or retarded cobra-venom hemolysis gave inconstant results with different sera.

### Summary.

1. We concur in the prevalent conclusion that the Much-Holzmann reaction has no diagnostic significance.

2. The influence which a given serum has on cobra-venom hemolysis is fairly constant.

3. The relation between the groups to which the serum and corpuscles belong has no influence on the result of the Much-Holzmann reaction provided the serum contains no isohemolysin for the corpuscles used.

4. There is practically no difference in the effect of active and inactivated serum on cobra-venom hemolysis.

5. Corpuscles from normal individuals belonging to different groups vary in their resistance to cobra-venom hemolysis to a very slight extent.

6. The action of different sera in favoring or retarding cobra-venom hemolysis is inconstant.

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